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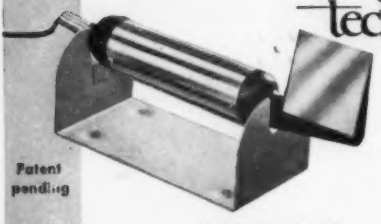

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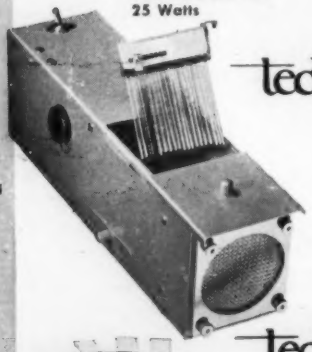

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Conference on Fungicides

FUNGICIDES and their Mode of Action was the subject of a conference held November 24 and 25 at the National Research Council. The conference was called by the Prevention of Deterioration Center, a council agency, in response to the growing feeling that work in this eld is largely empirical in nature and broad guiding principles are needed.

The conference opened with a discussion of growth and spore germination as a basis for testing (J. G. Horsfall, Connecticut Agricultural Experiment Station), and of factors controlling spore germination in specific instances (P. J. Allen, University of Wisconsin). It was brought out that many growth inhibitors do not affect spore germination, and vice versa. The arrested growth state typical of spores is apparently due to rather specific enzyme blocks, and in one case to a volatile inhibitor. Although further study of spore germination is important, it is clear that the results of evaluation tests will vary with the principle used. Tests based on the weakening of cellulose fibers may well involve both factors, since the fungus is usually inoculated as spores, and the extent of attack will roughly parallel the growth. The test organism must be chosen with full appreciation of the specificity of response sometimes shown. Thus *Botrytis* is 100 times more sensitive to dimethyldithiocarbamate than *Aspergillus niger*, yet both are equally sensitive to trichlorophenol (H. L. Klopping, Du Pont).

Although toxic substances are generally considered to act on specific enzyme systems, little attempt has yet been made to view the action of fungicides in this way. An important step in this direction was taken at the conference by considering enzyme inhibitors. D. E. Green (University of Wisconsin) defined three major categories of enzyme inhibition: (1) by resemblance to the substrate (e.g., malonic and fluorocitric acids and the antivitamins), (2) by modifying an enzymic prosthetic group (e.g., HCN, CO, and azide), (3) by modifying the protein moiety of an enzyme (e.g., sulfhydryl reagents).

By the end of the conference it developed that most of the known fungicides fall into one or the other category. In discussing the activity of chelating agents, conditions were described in which these agents can activate enzyme systems by altering their colloidal state rather than by combining with metals (W. D. Bonner, Jr., Smithsonian Institution). Other systems were described in which chelating agents cause an inhibition reversible by specific metals (W. J. Nickerson, Rutgers University). These, of course, are clear examples of inhibition by combining with a metal, even when that metal is in relatively stable complex form, for the stability constants of metal-chelates like copper oxine are very high. However, the remarkable fact that the fungitoxicity of oxine is exceeded by that of its copper complex remains unexplained. The behavior of halogen-substituted derivatives of oxine shows that these are less active at neutral pH, but much more so at high acidities, where copper-oxine is appreciably dissociated (S. S. Block and E. G. Kovach, University of Florida).

In the important group of substituted phenols it was pointed out that *ortho*-substitution, especially with normal- or cyclo-alkyl groups, always increases fungicidal activity. Some of the puzzling effects of substitution on activity parallel those on the dissociation of the phenolic hydroxyl (H. Shirk, National Research Council). Similar effects are seen in the bis-phenols, the fungicidal activity and stability of which are on the whole greater (P. B. Marsh, U. S. Department of Agriculture).

Among the acrylates, many compounds have been quantitatively compared (C. Wessel, NRC). A discussion of the basis of their action (L. P. Hammett, Columbia University; R. C. Elderfield, University of Michigan) led to the conclusion that it may depend on combination with thiols.

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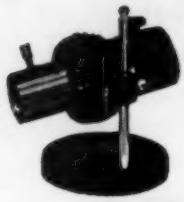

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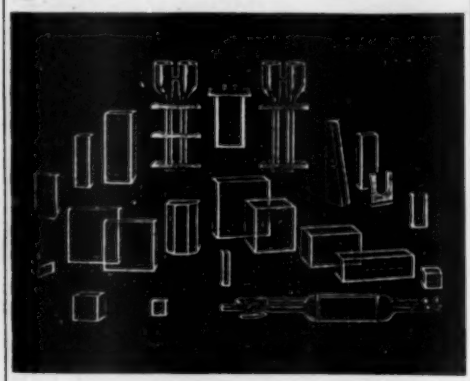

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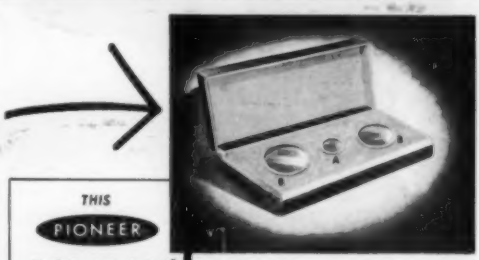

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The Problem of Experimentation on Human Beings[1]

I. The Research Worker's Point of View

Michael B. Shimkin

University of California School of Medicine, San Francisco

IT IS CONSIDERED AXIOMATIC that the purpose of medical research, perhaps in contrast with more general research, is to discover, improve, or extend information regarding man, his functions, and his relationships to his environment. It follows that a primary scientific criterion of usefulness in medical research is whether the observed phenomena can verily be produced in, or applied to, human beings. Findings on other species may have general or specific validity for man, but the ultimate establishment of such validity must rest in each instance upon direct observations on man. At some point in any medical research, therefore, the investigation must be performed with human beings, if that research is to fulfill its primary objective.

Despite these obvious considerations, and despite the demonstrated value of medical research in terms of saving life, relieving pain, and achieving other goals considered worthy, the use of human beings for experimental purposes often encounters vigorous opposition. Proposal of such investigations, even to groups trained in scientific disciplines, may result in outright rejection or in the suggestion that animal experiments that a priori can be seen to be inadequate for the solution of the problem be substituted.

Analysis of the reasons for these attitudes is essential if experimentation on human beings is to be pursued on a scale commensurate with its importance. In my opinion, three primary considerations are involved.

The first is the basic question as to what constitutes an experiment. An experiment is a sequence resulting from an active determination to pursue a certain course and to record and interpret the ensuing observations. Hence, to do nothing, or to prevent others from doing anything, is itself a type of experiment, for the prevention of experimentation is tantamount to the assumption of responsibility for an experiment different from the one proposed. As much knowledge and as weighty reasons are required for one course of action as for the other, and it should be demonstrated that the proposed experiment is more dangerous or more painful than the known results of inaction.

The second consideration is that medical experimentation on human beings, in its broadest meaning and for the good of the individual patient, takes place continually in every doctor's office (*1*). Hence the general question of human experimentation is one of degree rather than of kind. Deliberate experimentation on a group of cases with adequate controls rather than on individual patients is merely an efficient and convenient means of collecting and interpreting data that would otherwise be dispersed and inaccessible. A specific study may still be rejected because of its hazards, expense, or relative lack of utility, but at least it should not be rejected outright simply because it is an experiment on human beings.

The third consideration is that of the nature of medical responsibility. The responsibility of the individual physician to an individual patient has been clearly defined, maturely considered, and almost universally accepted; it has been tried and found good. Deliberate experimentation would seem to introduce a break with the accepted type and a replacement by forms of so-called responsibility, which should be deeply and rightly distrusted—the sort of thing that is called the duty of scientific man to society and the obligation of individuals to the race, under which all sorts of monstrosities have been practiced in absolutist states. But abuse does not preclude use. Responsibilities do exist, and it is better to define them and see that they are not abused than to deny their existence and to accept the consequences of denial. There is no basic difference between the experiment on an individual patient and the extension of the experiment to a group of similar patients. Both the physician and the patient, in fact, benefit from the increased facilities for consultation and comparison, stronger checks, and the accelerated collection of data. Here, again, a proposed experiment may be rejected on adequate grounds, but not merely because of general condemnation of the type of responsibility involved.

It is in the deliberate mass-experimental situations on man that traditional, unanalyzed attitudes make scientific studies inordinately difficult to initiate and to perform. And because of the absence of analysis, investigations that do not achieve diagnostic or therapeutic goals may remain unreported, to the detriment of further research and the repetition of futile procedures. Similarly, the untoward results of a procedure may be deleted from a published report in order to reduce possible criticisms of the study.

Research on human beings, of course, involves

[1] Based on a symposium held at the Medical Staff Conference, Oct. 10, 1951, Division of Medicine, University of California School of Medicine, San Francisco.

unique hazards, precautions, and responsibilities. Whenever human beings are to experiment on human beings, the mores of human conduct, including ethical, religious (*2*), and legal (*3*) considerations, cannot and must not be ignored or minimized.

What, then, are the proper rules of conduct that can be utilized in judging whether human beings should be involved in experimentation? Perhaps the clearest formulation of such rules was made at the Nuremberg medical trial (*4*). In its decision rendered on August 19, 1947, the Tribunal stated:

1. The voluntary consent of the human subject is absolutely essential. This means that the person involved should have legal capacity to give consent; should be so situated as to be able to exercise free power of choice, without the intervention of any element of force, fraud, deceit, duress, overreaching, or other ulterior form of constraint or coercion; and should have sufficient knowledge and comprehension of the elements of the subject matter involved as to enable him to make an understanding and enlightened decision. This latter element requires that before the acceptance of an affirmative decision by the experimental subject there should be made known to him the nature, duration, and purpose of the experiment; the method and means by which it is to be conducted; all inconveniences and hazards reasonably to be expected; and the effects upon his health or person which may possibly come from his participation in the experiment.

The duty and responsibility for ascertaining the quality of the consent rests upon each individual who initiates, directs, or engages in the experiment. It is a personal duty and responsibility which may not be delegated to another with impunity.

2. The experiment should be such as to yield fruitful results for the good of society, unprocurable by other methods or means of study, and not random and unnecessary in nature.

3. The experiment should be so designed and based on the results of animal experimentation and a knowledge of the natural history of the disease or other problem under study that the anticipated results will justify the performance of the experiment.

4. The experiment should be so conducted as to avoid all unnecessary physical and mental suffering and injury.

5. No experiment should be conducted where there is an *a priori* reason to believe that death or disabling injury will occur; except, perhaps, in those experiments where the experimental physicians also serve as subjects.

6. The degree of risk to be taken should never exceed that determined by the humanitarian importance of the problem to be solved by the experiment.

7. Proper preparations should be made and adequate facilities provided to protect the experimental subject against even remote possibilities of injury, disability, or death.

8. The experiment should be conducted only by scientifically qualified persons. The highest degree of skill and care should be required through all stages of the experiment of those who conduct or engage in the experiment.

9. During the course of the experiment the human subject should be at liberty to bring the experiment to an end if he has reached the physical or mental state where continuation of the experiment seems to him to be impossible.

10. During the course of the experiment the scientist in charge must be prepared to terminate the experiment at any stage, if he has probable cause to believe, in the exercise of the good faith, superior skill, and careful judgment required of him, that a continuation of the experiment is likely to result in injury, disability, or death to the experimental subject.

Much the same rules in regard to medical experiments on human beings have been delineated by the American Medical Association (*5*) and by the Green Committee (*6*) on the use of prisoners in investigations. Analysis of the rules shows that they can be reduced to two primary principles: First, the investigators must be thoroughly trained in the scientific disciplines of the problem, must understand and appreciate the ethics involved, and must thus be competent to undertake and to carry out the experiment. Second, the human experimental subject must understand and voluntarily consent to the procedure, and must not be selected upon any basis such as race, religion, level of education, or economic status. In other words, *the investigators and the subjects are human beings with entirely equal, inalienable rights* that supersede any considerations of science or general public welfare. Finally, research on human beings is too hazardous and implies too many responsibilities to be undertaken by lone investigators. It should be a group effort supported by a proper consultative body. Experimentation even on oneself without such collaboration and consultation seems as indefensible as similar experimentation on another individual.

Once these primary principles are accepted, the application of the stated rules to any specific situation involving human experimentation is modified by several factors and is relative to these factors. The factors can be divided into two classes: the physical and mental condition of the prospective subject, and the type of experiment contemplated.

1. *The condition of the subject.* Human subjects of medical investigations can be divided into three categories:

a) *The normal subject.* The rules of conduct governing research on human beings basically stress and primarily refer to experimentation that may be carried out on normal, healthy individuals. It is here that the most thorough information must be available from animal studies and from clinical studies on patients before experimentation can be considered as justified. It is obvious that opinion regarding the thoroughness and completeness of the preliminary studies will vary widely, and it will be modified by the importance and the immediate nature of the problem. The best final criterion for the justification of the use of normal individuals is the voluntary participation of the experimenters themselves as the subjects.

b) *The subject with reversible disease.* Another class of persons who may participate in an investigation is one with a reversible or nonfatal disease. It is rather evident that these individuals should ordinarily be involved in research only after data are obtained on animals or on patients with more serious conditions which show that the procedure has promise of curing the disease more quickly, reducing more serious complications, or affording more complete symptomatic

relief. In other words, the experimentation is more or less limited to research in therapy, and even here the hazards of the drug or procedure must be weighed against the hazards of the disease in each instance and individual (*7*).

c) *The subject with fatal disease.* The third group of human beings that can be considered for experimentation are patients with incurable, inexorably fatal afflictions. No better example is afforded than by patients with advanced neoplastic disease. Although palliation still remains the first consideration in proper medical management, an unparalleled opportunity exists for the wider study of these individuals in pharmacologic, physiologic, and other medical investigations. It is the experience of many physicians that this type of patient often wants, and even demands, that something be done for advancement of knowledge if not for personal benefit.

2. *The type of experiment.* Research on human beings can also be divided into three categories, dependent upon the type of investigation contemplated.

a) *"Passive" research.* In this group fall investigations that use human tissues or products that are separated from the individual. Typical examples are biochemical determinations of enzyme reactions on tissue specimens or the measure of amino acids in blood. The investigation may be initiated on human tissue or may be tried on human material after previous determinations on biological systems of animal or plant origin. Unless it involves a deliberate surgical operation or the maintenance of the human subject under special conditions before the material is obtained, the role of the human being is a passive one and does not include actual participation in the research.

b) *"Late active" research.* In this type of research, investigations on man are undertaken after information is available from comparable studies in animals. Typical examples are afforded by the pharmacologic evaluation of new drugs (*8*). On the basis of *in vitro* and animal studies that show a certain chemical to cure a certain infection, and after comprehensive determinations of the toxic effects on animals, cautious clinical trials must be initiated somewhere and by someone to demonstrate whether the chemical is in fact effective and safe for human use. New diagnostic procedures and new surgical operations also fall in this category. The human being is an active participant in the research but enters it at its final or late stage.

c) *"Initial active" research.* Certain investigations may be undertaken first, or exclusively, on human beings. Examples of this class of research imply that the study can be pursued best, or only, directly on man. Experiments on the apposition of the thumb can hardly be performed on any but a human hand. Many disease processes cannot be, or at least up to now have not been, successfully reproduced in animals. In other instances the occurrence of an abnormality is so rare and difficult to find in animals that no study except on human beings is practicable. Investigations that necessitate cooperative, complex psychologic reactions, which are either lacking or not comprehensible in animals, also require active initial participation by human subjects.

This is the category of research in man that is most difficult and complex in its ethical and other ramifications and implications. It is here that therapeutic or diagnostic indications may be unfounded or nonexistent. It is here, also, that fundamental physiologic or psychologic information of immediate application and import to man may be obtained.

The complex problem of the use of human subjects in medical research involves many suprascientific considerations. The guiding principles that have been developed would have general acceptance within the framework of our culture. The application of these principles to specific experimental situations, however, is still open to individual interpretations and differences. It is appropriate to indicate in this connection that science per se is neither moral nor immoral; it becomes moral or immoral only as moral or immoral human beings use its powerful techniques.

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II. The Physician's Point of View

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I THINK, from Dr. Shimkin's comprehensive and lucid presentation, it has become crystal clear—if it was not agreed to before—that experimentation on human beings, including the sick, must be performed if we are to advance our conquest of disease. There can be no question of the value of this type of investigation for the improvement of the care of the sick, and the fact is that experimentation on human beings is nothing new. It has taken place, is taking place, will take place, whenever physicians are in the process of introducing new diagnostic and therapeutic procedures or agents—new in general or new for the patient—or whenever investigations on the sick are being made that are of no immediate value

to them but are made to confirm or disprove some doubtful or suggested biological generalization.

Recently, however, the latter type of experimentation has become more and more extensive. At least three developments are evolving at present that seem to call for an appraisal from the physician's point of view; for it is predominantly physicians who are the experimenters, and it is the patient-physician relationship, in the broadest sense of the term—including the patient's and physician's attitudes toward life—which is deeply affected by these developments. The historical developments to which I refer are as follows: (1) The application of force, as in experiments performed in the insane asylums and concentration camps under the National Socialistic regime in Germany; (2) The frequent recourse to "hopelessly incurable" patients; (3) The increased technicalities of many problems and procedures.

The recent literature on the topic in this country has been contributed mainly by A. C. Ivy (*1*), Willard L. Sperry (*2*), and W. B. Bean, who published a perceptive article after this symposium was held (*3*). In Germany A. Mitscherlich and F. Mielke (*4*), and V. von Weizsaecker (*5*) wrote on it most penetratingly in connection with the well-known Nuremberg trials in 1947 against twenty National Socialistic physicians. Weizsaecker's publication shows particular insight into the problem, and I shall make further reference to it.

To be more explicit, some remarks are in order about (*a*) the type of experimentation we are discussing, (*b*) the technical necessity that physicians carry out the experiments, (*c*) the patient-physician relationship.

a) The type of experiments with which we are concerned is of no immediate value to the patient but is made to confirm or disprove some doubtful or suggested biological generalization. We assume, of course, that all types of experiments discussed here have a common denominator—a physical or chemical relationship, and, in this sense, all may be considered to be of value to the individual experimented on. However, to the person under observation there are significantly different encroachments in an experiment. In the words of Hippocrates, "Art is long, but life is short," and not all experiments performed on men will ever be of value to these particular men. And it is with these breathing men that we are concerned as physicians.

b) There is no legalized profession of experimenters on human beings outside the medical profession, and no other group is privileged to perform any but the simplest somatic experiments on human beings. Paragraphs 2137 and 2141 of the Business and Professional Code of the State of California cover this subject, and it should be noted that the Principles of Medical Ethics of the American Medical Association (*6*) nowhere forbids experimentation. As a matter of fact, experimentation for the patient's immediate good forms an integral part of the physician's care of his patients; and experiments to confirm or disprove a biological generalization with regard to man certainly cannot be better performed than by the profession that is trained more completely than any other in comprehending the somatic and psychological aspects of human life, be it healthy or diseased. There is another reason why the medical profession is so uniquely privileged. It is closely related to what we shall call the *original* patient-physician relationship.

c) The patient-physician relationship is fully understood only when viewed from two aspects. The relationship of the one who performs experiments of no immediate value to the person under observation is impersonal and objective because of the character of the research. Experimentation is the basis on which the two meet, the original bond between them. The fact that the experimenter is a physician is, from this point of view, almost incidental, although he occupies this position because of the legal technicalities mentioned above and because of his special training. At the same time, the person experimented on is studied solely because of the special set of symptoms he presents. He is sick in the objective sense—i.e., he is different from the healthy man as measured against some common standard, just as one piece of ore may be different from another. He is indeed an object in the grammatical sense, too. The physician, like any experimenter in the field of physics and chemistry, is the subject, for it is he who does the experimenting. But even though he is the subject in the grammatical sense, he is not the subject in the real, personal sense. Every effort is made, and must be made, to depersonalize him and to eliminate every subjective factor. Invoked by the drive for generalization and specialization, objectivity is the password throughout.

Obviously, there exists a second, quite different, relationship between a physician and a sick person. Historically, it is the *original* and, indeed, the basic justification for our profession. Here one human being is in distress, in need, crying for help; and another fellow human being is concerned and wants to assist him. The cry for help and the desire to render it precipitate the relationship. Here *both* the healthy and the sick persons are subjects, fellow-companions, partners to conquer a common enemy who has overwhelmed one of them. Theirs is a relationship between two "I's," like that between lovers, friends, pupil and teacher (*4*). I have called it "mutual obligations of equals," or, to use Weizsaecker's term, it is "solidarity." Objective experimentation to confirm or disprove some doubtful or suggested biological generalization is foreign to this relationship. It is not the point of contact between the two partners, for it would involve taking advantage of the patient's cry for help, and of his insecurity. Moreover, in his role as a fellow human being, the physician is only too keenly aware that all our lives are limited, one-way trips, and that death per se, despite its absence from physiological texts, is a physiological phenomenon, befalling us all (*7*).

Still another problem related to the aspect of man as a subject is pertinent to our discussion. Noting

that "man finds himself in the peculiar situation of being a finite creature and yet gifted to survey eternity," the theologian Reinhold Niebuhr has expressed it in the statement: "Self intrudes itself into every ideal when thought gives place to action" (*8*). Marx and Freud are perhaps the best-known modern authors who have implemented this thought and challenged the rationalistic faith that action depends on conscious reasoning alone and is not conditioned by the egotism of our finiteness, although one should add, perhaps, that Christian dialectics penetrate much deeper.

It may be concluded that the relationship in experimentation between experimenter and experimented-on, entered upon not to help but to confirm or disprove some doubtful or suggested biological generalization, is impersonal and objective. The *original,* basic patient-physician relationship implies the concept of solidarity, of life's finiteness, of man's indigenous selfishness. Experimentation as just described is foreign to it.

The language differentiates between the objective "being-sick" and the subjective "being-a-patient." It does not differentiate these two aspects with regard to the term "physician," although one may differentiate between the two facets of the physician as the physician-experimenter and the physician-friend.

Against this background let us view the recent extension of experimentation as illustrated by the three developments mentioned early in these remarks.

1) *The application of force.* Although the question is more intricate than it may seem at first sight, apparently no problem exists in American culture. I think the overwhelming majority of physician-experimenters, if not thoroughly aware of the nature of the original patient-physician relationship, are so deeply rooted in the democratic spirit that they agree, and will continue to agree, that the use of force is not justified on a single person, even if millions of other lives could be saved by such an act. They realize that the act would not just save millions of lives but that, as an amoral act from the standpoint of democratic brotherhood, it might create millions of amoral sequels, and that the moral history of mankind is more important than the scientific.

2) *The problem of the "hopelessly incurable."* This question illustrates the chaos that results when the two aspects of the patient-physician relationship are not clearly recognized, and no conscious attempt is made to separate the two. All—the science of medicine, the suffering patient, the physician-experimenter, and the physician-friend—lose by this confusion. The literature suggests that the classification of persons as "hopelessly sick" is not intended to be merely a presentation of fact in the objective sense on the part of the experimenter, but, by its characterization as "hopeless," is intended to justify an experimenter's self-permit for greater boldness. It is an apparent attempt to express a conscious effort of restraint on his part when, in performing experiments that endanger the lives of the experimented-on sick, the experimenter restricts himself to those "marked by death." It is meant to be noble in the democratic spirit, yet it unconsciously challenges this spirit more subtly but no less than the use of force, because it violates the concept of equality or brotherhood in violating the principle of the original patient-physician relationship. From the experimenter's point of view, the description "hopelessly incurable" is not germane to his purpose. The designation is inadequate, because it does not specify the time element—hopeless within hours, days, months, years? And, if months or years are concerned, do all experts agree on the status of their respective sciences and deny the possibility of discovering effective agents within such a period? The term is also unnecessary, for unless he wants to study mechanisms of death, it does not make any difference to the experimenter whether the experimented-on is hopelessly affected by the disease under observation.

From the standpoint of the physician-friend, the assertion is not germane to his purpose, either. To him it is an expression of detachment between physician and patient, the announcement of a scale of partnership *vs.* domination quite contrary to its original spirit. As a matter of fact, it creates the paradox that the healthier the patient, the more he should be the concern of his physician; the sicker, the less. From the standpoint of solidarity we all are finite individuals who have to die; to restore or preserve health is the physician-friend's concern, not to trespass presumptuously upon our inevitable "mortal lot." Whatever other requests for adjustments our society may make of the individual (except for the soldier, who holds a unique place), our culture does not, under any circumstances, intend to endanger individual life.

There are, as Weizsaecker points out, two aspects concerning the "hopelessly sick" that emerge from the concept of a true patient-physician relationship—the aspect of pity and the aspect of sacrifice. Dr. Shimkin also mentioned the latter. I follow Weizsaecker when he says that pity, as such, does not imply the spirit of solidarity. It is not pity that the patient wants, but help. Selfish motivation, entirely unconscious, may enter in the guise of pity; and, on the other hand, conscious selfishness—for instance, the physician's greed for money—may help a patient more than the physician's pity. A second justification for experimentation on the "hopelessly sick" on the basis of the original patient-physician relationship would be the patient's wish to sacrifice himself, similar to that of a conscientious objector. The physician-friend who wants to experiment might most humbly accept such a wish as the highest form of human transcendence, being aware that true equality does not exist under such circumstances, for the offering of one partner happens to coincide with the self-interest of the other. Experimentation on the so-called hopelessly sick requires, therefore, a tremendous amount of self-criticism, self-discipline, and understanding of life's essential attributes, lest it be perverted to unconscious barbarity. The phrase needs to be eliminated, and an objective and more precise terminology used instead.

3) *The increased technicalities.* It is obvious to

every physician, be he an experimenter or a physician-friend, that, even in the field of daily practice, assertions that, following explanations of the seriousness of an operation and the nature of the patient's disease, it was "agreed" between patient and physician to operate, can be true only in the vaguest sense of the term "agree." The agreement refers only to acts of commission and not to those of omission, and one has only to think of present-day specialization in medicine in order to realize that the patient is frequently not able to grasp all the implications of a certain procedure so far as his health is concerned. Only the spirit of the original patient-physician relationship, faith on the part of the patient and the attitude expressed by the Hippocratic Oath, can bridge this gap. How much greater is this difficulty in an experimental procedure, where selfishness plays a role?

Present types of experimentation on the sick clearly challenge tremendously the basic concepts of the original patient-physician relationship. All the encroachments imposed by society upon this relationship, such as reporting certain diseases, requesting certain types of inoculations, evaluating fitness for work or right to compensation, shrink before the challenge the profession itself raises. Yet Dr. Shimkin has pointed out—and we all agree—the great significance of human experimentation for the cure of the sick. We are facing a dilemma which can never be decided in final terms but only on a temporal basis.

Perhaps a glance at the way the legal profession meets the moral and technical demands of society and the individual when a conflict arises between the two will offer a cue to a solution of our problem. As we all know, that profession provides each of the two with a representative of equal stature: there, the prosecuting attorney, and here, the defense attorney. Similar arrangements may have to be developed in the field of human experimentation, performed not for the good of the individual patient but made to confirm or disprove a doubtful or suggested biological generalization. Research and care would not be pursued by the same doctor for the same person, but would be kept distinct. The physician-friend and the physician-experimenter would be two different persons as far as a single patient is concerned—for instance, my patients would become research objects for someone else, and I would be permitted to experiment only with the patients of another physician. The responsibility for the patient as patient would rest, during the experimental period, with the physician-friend, unless the patient decided differently. Retaining his original physician as personal adviser, the patient would at least be under less conflict than he is at present when the question of experimentation arises.

With reference to increasing technicalities, the forms that patients must sign when about to volunteer for experimentation, or even to undergo an operation, might be so phrased as to state not only the patient's consent, but also the physician's affirmation of his utmost effort to protect the patient from harm and of his most careful judgment in deciding on an operation. Under those circumstances the obligations of the profession toward the individual and society would not be blurred.

The problem we face thus presents a true dilemma, being tragic in the classical sense; both its aspects are of equal value in thought, and a course of action must be decided anew for each actual situation, because the varieties of actual situations are as infinite as history itself. A given situation may demand that the attitude of the physician-experimenter and that of the physician-friend be embodied in one person. Unless we recognize the basic differences of the two attitudes, each will suffer, as demonstrated by the confused concept of the "hopelessly incurable;" or one will be needlessly neglected, as demonstrated by our failure to supplement forms requesting consent from a patient with some corresponding affirmation of utmost concern for his welfare on the part of the attending physician.

It is not the conquest of nature but the re-evaluation of man that appears to be the basic problem of our times. It is the re-evaluation of man as—to express it in old yet valid terms—"created in the image of God and tempted by the devil," not as a replica of innocent beasts, which, however cruel, cannot commit any crime. We must be alert with ourselves lest, in our zeal for truth, we create healthy bodies at the cost of morally dulled minds.

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III. Limits of the Right of a Person to Consent to Experimentation on Himself

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MEDICOLEGAL CONFERENCES are, I think, usually unsatisfactory from the legal side. The lawyers who know the subject best generally dodge the responsibility and leave it to someone like myself to rush in where they fear to tread. There is a reason why the lawyer dislikes to express an opinion. The Anglo-American lawyer wisely refuses to commit himself on a suppositious case. The courts refuse to render advisory opinions. They will decide only an actual case presented to them by the parties in the regular way. You may say, "Well, I am asking you now, 'Can I do this?'" To which I reply, "Is this an actual case in which you are about to take action?" In an actual case, you have all the facts, and it is the concrete situation and not the abstract theory that dictates the decision. If it is a question of experimental treatment of a real person, all the considerations presented by Dr. Shimkin are relevant and affect the conclusions. The rules referred to by Dr. Shimkin should be memorized and applied in every case by doctors and research workers. Even then the lawyer cannot guarantee his answer, any more than a doctor will guarantee a cure.

In general, a medical man may not treat or operate on a human being without his consent. It makes no difference that the person needs treatment or will die if he does not get it. If the consent has not been obtained, it is no defense that the operation was skillfully performed and saved the patient's life. Where the patient is unconscious and needs immediate treatment, it can be done; but even there, if the husband or wife is present, his or her consent may be necessary. Consent to an appendectomy is not consent to a gall bladder operation. In practice the surgeon gets a complete authorization, even if the operation is for what appears to be a minor condition, because what seems to be superficial may turn out to be serious. But this does not solve all the questions. Consent itself, even if written, may be effective only if there has been a complete disclosure. The lay patient cannot understand the process of treatment, but the possible end results must be made clear to him. I doubt whether the printed forms of consent are adequate in some cases. The patient may claim, perhaps correctly, that he did not understand what was meant.

In many medical cases this consent is not obtained. There are difficult diseases of which little is known. New drugs are coming out with bewildering rapidity, and enthusiastic discoverers give optimistic accounts. Physicians try the drugs on patients, commonly without informing them. It is not practical to get a written waiver in advance, or each time an experimental drug is used. Death, blindness, and serious injuries have sometimes resulted from treatment—more often than we may realize. Some modern drugs cure a disease but produce serious side effects. On the other hand, brilliant medical men or their insurance carriers have had to pay damages for unsuccessful treatments where the usual but unsatisfactory procedures had not been followed. This discourages progress. The opinions of some courts read as if the judge seemed to think that there is a prescribed cure for every ailment of the patient. As a matter of fact, in a large number of cases, the doctor must experiment to make the diagnosis, as well as to carry out the treatment. Each person is apt to present an individual case, in some respects not conforming to general rules.

This kind of experimentation—if it can be called experimentation—is clearly not the kind condemned by the law. Where, however, there is a clear-cut case in which there is a well-recognized treatment, but the doctor has discovered what he believes to be a better treatment, the law puts the risk on the doctor if the treatment fails. As one court put it, "We have little doubt that, if the first case of vaccination had proved disastrous and injured the patient, the physician should have been held liable." It seems unfair to put such a risk on the doctor. The above quotation was not necessary for the decision. The cases that have actually come before the appellate courts have been actions against quacks and charlatans. None involved a real scientist observing the proper precautions.[2]

Practically, there is not much danger where sound rules for experimentation are observed: careful preliminary study by a well-trained man, animal experimentation where possible, desperate remedies resorted to only in hopeless cases, treatment stopped at the first danger sign. In the exceptional case the doctor can protect himself by full disclosure and the written consent of the patient. The rules laid down at the Nuremberg trial might well be taken by the courts as the standard by which the doctor should be judged. The malpractice suit is a headache to the medical profession, but some doctors say that, if it were not the law, doctors would have to make it the law, for it is a powerful stimulus to keep the doctor on his toes, up to date, and careful.

There is the fundamental difficulty to which Dr. Guttentag has called attention. It is the business of the doctor to do his best for his patient. Research on the patient for purposes other than his welfare has no place; yet, if the doctor is not also a research man, he is not the best kind of doctor, and if the research specialist is not interested in the welfare of the patient entrusted to him, he is not the best researcher.

[2] The cases are examined in *Calif. Law Rev.*, **40**, 159, and in *Ann. Western Med. and Surg.*, **6**, 164 (Mar. 1952).

Americans who studied at Vienna, Leipzig, and other medical centers in Europe before the first world war admired the skill of the doctors but were shocked at treatment administered and operations performed which would never have been undertaken had the welfare of the patient been the sole consideration.

Although there is no legal authority, and consent is theoretically necessary, it may be that, on the maxim *"De minimis non curat lex"*—"The law does not regard trifles"—an extra drop of blood to build up a control group for a research study, or the use of tissue that has been properly severed would not be condemned by the court. But the medical profession should consider carefully whether, as a matter of good public relations, nothing should be done to a patient except for his benefit, and whether he should not be used either directly or indirectly as a guinea pig without his consent. A doctor who has the reputation of experimenting with his patient is avoided by those who know that reputation.

That brings us to the question of experimentation on persons—with their consent—not for any disease and not for any direct benefit to the patient, but solely for the advancement of science. How far can one consent to serious injury to himself? The analogies are not close. Abortion, except for therapeutic reasons, is a crime, and the consent of the woman is no defense for the doctor. A person cannot legally consent to his own death; it is murder by the person who kills him. Societies in England and the United States are trying to legalize euthanasia for those suffering from incurable disease, and juries have sometimes acquitted a parent who has put a suffering child out of his misery. If it had been his dog instead of his child, he would have been punished for not killing it. In the case of the birth of monstrosities the doctor may perform the killing and no one be any the wiser, but legally it is murder. A person may not consent to a serious injury amounting to a maim. The classical case was the man who cut off his hand to make himself a more successful beggar. Injuries inflicted to avoid military service are not unknown, and are criminal.

Sterilization presents a more difficult problem. The late Lord Riddell seemed to condemn it without exception but, in the same volume, approved it for the feeble-minded. Statutes concerning the feeble-minded have been sustained. In this country what little authority there is seems to permit sterilization where death, insanity, or serious disability would result from pregnancy. Economic considerations based on the number of children are a doubtful basis, and it probably would not be sanctioned simply to avoid the possibility of having children.

These situations all involve serious injury inflicted by consent for the direct advantage of the one permitting it. The motive of the advancement of science presents a different case.

The use of prisoners, racial minorities, or religious groups for experiments resulting in death has been universally condemned. Yet the state drafts men for war and death. Perhaps Colonel Johnson will tell us whether men could be drafted to undergo dangerous experiments in order to save the lives of as many soldiers as possible. There are other hazardous occupations. No prosecution seems to have followed where antivaccinationists have been encouraged to receive smallpox germs, or religious fanatics to submit to snake bites. Although there are no cases, it could not be considered a crime of the experimenter where the highest public praise is accorded to those incurables who offer themselves for experimental purposes in order that persons may not have to suffer in the future as they have. The airmen who have died in pressure experiments to make air travel possible for others, the Walter Reeds who have risked disease germs to determine causation where animal experimentation has failed, and other martyrs to science who have missed success except in a negative way so that that particular experiment need not be repeated, are heroes.

Just a word on consent for those incapable of giving consent. Where the parent of a child refuses to allow treatment essential to save his life, the doctor cannot act. In some cases the matter has been brought to the court on the theory that the parents are not providing for the child. The child is declared a ward of the court, and the court can then give permission for the treatment.

We would probably agree that the poor, the aged, the insane, and the feeble-minded should not be guinea pigs for experimentation. Reasonable experiments to improve their condition under the rules laid down should be approved. Prisoners can usually give consent, but in all such cases great care should be taken to make sure that consent is not coerced.

IV. Civil Rights of Military Personnel Regarding Medical Care and Experimental Procedures

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THE RIGHT OF MILITARY PERSONNEL to refuse to submit to medical care and the limitation of their rights to volunteer or consent to experimentation in the medical field present several questions. It is obvious, of course, that a soldier has the right, the same as any other individual, to submit voluntarily to medical care provided by the military establishment for the purpose of cor-

recting any defect or curing any ailment with which he is afflicted. The important question, which is most germane to this discussion, is whether the military authorities can require him to submit to medical treatment for such defect or ailment, regardless of his choice in the matter. We all know that a civilian need not submit to medical treatment unless he so desires.

Paragraph 24, Army Regulations 600-10, 10 November 1950, provides that persons in the military service who refuse to submit to medical or surgical (including dental) operation, treatment, or diagnostic procedures will be examined by a board of three medical officers, and if, in their opinion, the operation, treatment, or diagnostic procedure advised or recommended is necessary to enable such a person to perform properly his military duties, and that the treatment will have such curative effect, the patient must consent to the treatment or be subject to disciplinary action, including trial by court-martial if warranted under the circumstances. All such cases must be referred to the Surgeon General for consideration and review prior to the institution of any disciplinary action.

The reason for the distinction between the civil rights of civilians and military personnel under such circumstances is, of course, based upon military necessity. Not only does a person in the military service have the responsibility to take all reasonable measures to remain physically qualified for duty, but it would be grossly unfair, particularly in time of war, to allow personnel to be relieved of service in the Armed Forces because of a physical defect that could be completely cured by operation or treatment.

Although cases arising in this field are rare, the Judge Advocate General of the Army has been called upon to render opinions in some instances. In order to understand fully the safeguards which, by judicial opinion and decision, are afforded to military personnel in this situation, it may be interesting to review briefly a few of the decisions:

1) In GCM 120385, it was held that inoculation or vaccination is not such "medical treatment" as is contemplated by Army Regulations, and refusal by military personnel to submit to this form of treatment is an offense under military law, without the necessity of obtaining the opinion of a board of officers or reference of the matter to the Office of the Surgeon General. It was stated that inoculation and vaccination are preventive measures for the protection of the health not only of the soldier himself, but of others, and are expressly required of every person entering the service.

2) In GCM 121820, a soldier was convicted for refusal to submit to a surgical operation for goiter. In reviewing the record of trial, the Judge Advocate General held that the evidence was legally insufficient to sustain the conviction because the accused was in a state of nervous instability, excitement, and fear resulting from pathological conditions arising from his ailment, and that it would be unjust in the last degree to punish the accused for declining to submit to an operation, when the very diseased condition from which he was suffering caused him to refuse to submit to treatment.

3) In GCM 125224, the accused was convicted by court-martial for refusal to submit to a surgical operation known as vesiculotomy. It appeared that the operation consisted of a particular kind of draining first used many years ago by the officer who recommended it in this case, and used to some extent by other physicians. It was not shown, however, that the particular operation or any similar operation had been universally accepted by the medical profession as a cure for the disease from which the accused was suffering. In determining that the conviction could not be sustained, the Judge Advocate General based his opinion on the fact that the regulation contemplates, as far as major operations are concerned, only those so thoroughly tried and generally used by the medical profession that they have definitely and finally passed the novel and experimental stages, and have been accepted as standard operations in surgery. Not only must this requirement be clearly met, but it must also be shown that such an operation is generally recognized by the profession as being a cure for the particular disease with which the subject of the operation is afflicted.

4) In GCM 156980, the Judge Advocate General stated that, to support a conviction of having refused to submit to certain medical treatments prescribed by the attending surgeon and alleged to have been necessary and without risk to the life of the accused, there must be evidence that the treatment was, in fact, necessary to make the accused physically fit to perform his military duties.

5) In a court-martial case arising during World War II, a soldier refused to submit to certain dental treatment, including oral surgery, for an ailment the existence of which rendered him unfit for overseas or combat duty. The board of officers concluded that the operation recommended by the attending surgeon was a very simple procedure, universally accepted by the profession as a cure for the condition of the accused. In sustaining the conviction, the Judge Advocate General held that all requirements of the regulation had been complied with, the operation was one universally accepted by the profession as a cure for the ailment, hence the accused, as a person in the military service, had committed an offense against the Articles of War in refusing to submit to the treatment.

Briefly summarized, I think the foregoing regulations and decisions thereunder require military personnel to submit to medical treatment for existing ailments that render them unfit for further military service, provided: (*a*) the board of medical officers convened pursuant to the regulation and the Surgeon General concur in the diagnosis; and (*b*) the treatment or operation is one generally accepted by the profession as a complete cure for the ailment or disease with which the accused is afflicted.

The right of military personnel to volunteer for experimentation on themselves is a field in which few, if any, actual precedents exist. Paragraph 12, AR 600-10, reads as follows:

There are limitations upon the activities of officers and other personnel subject to military law. The general principle underlying such limitations is that all members of the Army Establishment, when subject to military law, are bound to refrain from all business and professional activities and interests not directly connected with their military duties which would tend to interfere with or hamper in any degree their full and proper discharge of their duties or which would normally give rise to a reasonable suspicion that such participation would have that effect. Any substantial departure from this underlying

principle will constitute conduct punishable under the Uniform Code of Military Justice.

Although this paragraph does not specifically refer to the exact topic under discussion, it is, nevertheless, sufficiently broad in its terms that I believe it is safe to say that a soldier is prohibited from consenting to any form of experimentation upon himself which would "interfere with or hamper in any degree his full and proper discharge of his duties, or which would normally give rise to a reasonable suspicion that such participation would have that effect." Although this would not necessarily be considered "misconduct" on the part of military personnel, it certainly would be considered "not in line of duty" if undergoing such experimentation incapacitated the man in any degree for the performance of his military duties. As a practical matter, questions of this kind are considered in Army terminology as "matters of command." I mean by this that it is possible for military personnel who voluntarily desire to undergo experimentation upon themselves to obtain permission from competent authority with assigned powers to grant such requests. As a matter of fact, we are all cognizant of the results obtained by Walter Reed in Cuba in the early 1900s when, assisted by volunteer military personnel, he was able to determine the cause of, and initiate successful preventive measures in, the yellow fever epidemic. That comparable experimentation and research in various fields are continuing at this time is apparent.

The Department of the Army has issued Special Regulation 70-30-1 24 January 1949, Subject: Research in Human Resources and Military Psychology. This regulation sets forth the interest of the Army in basic and applied research in the field of human resources and military psychology, and attempts to establish organizational policies and responsibilities for supervision, official channels of communication, and coordination for such programs. The regulation identifies the responsible agencies in the military establishment and the fields of interest in this subject. Authority is granted to contract with responsible agencies for research after study at Department of the Army level determines the necessity and probability of the results to be achieved.

The fields of research enumerated in this Special Regulation, for which the Medical Department is designated as the responsible agency, are as follows:

1) *Psychophysiology and psychopathology*
   - *a*) Sensory discrimination and perception
   - *b*) Psychomotor functions and motor skills
   - *c*) Biomechanics and human engineering
   - *d*) General and special organic conditions (including tension, shock, deprivation, motivation, emotions, fatigue, sleep, glandular conditions, and nutrition)
   - *e*) General and special environmental conditions (including temperature, humidity, ventilation, noise, etc.)
   - *f*) Analysis of adjustment levels and mental disorders for purposes of prevention, diagnosis, therapy, and rehabilitation

2) *Training research*
   - *a*) Adjustment and rehabilitation methods (including personnel counseling techniques, psychotherapeutic methods, and the sociopsychological rehabilitation of the physically handicapped)

All proposals for research projects in these fields that are of Army-wide interest will be transmitted to the Office of the Surgeon General for allocation. Initiation of, or contracting for, such projects will take place only after study and authorization by the Medical Department, and with such coordination and authorization as directed for the purpose of proper allocation, and for the achievement of the necessary balance between research and development programs. Every effort will be made to set up a strong and well-integrated program of research in human resources and military psychology.

Although this regulation is, of course, primarily concerned with fields of research and experimentation in which the Army is interested, it also indicates that, if such a program involves experimentation on the human body, authorization for the use of volunteer military personnel as subjects could be obtained. Needless to say, the Medical Department would not receive volunteers in this field if it considered the experimentation unduly hazardous or unnecessary.

One project in this field, which has been approved by the Medical Department of the Army and is now in operation, is treatment, experimentation, and research in burn cases. The procedures implementing this program, set forth in a recent circular from the Surgeon General's office, can be summarized as follows:

1) A burn study project was established approximately two years ago at the Surgical Research Unit, Brooke Army Hospital, Brooke Army Medical Center, Fort Sam Houston, Texas. It is expected that this project will be continued and expanded if adequate numbers of cases are available for study. Cases suitable for study are considered to be those severe burns in excess of 10 per cent of body surface involvement. The facilities available at Brooke Army Hospital for the treatment and study of these cases are outstanding.

2) Accordingly, severe burn cases may be transferred to Brooke Army Hospital for observation, treatment, and study when it is determined that they are transportable and are appropriate cases for treatment at that hospital. In the interest of expeditious movement of these patients, direct communication with the Commanding General, Brooke Army Medical Center, is authorized to determine availability of a bed and whether the case is appropriate for transfer. After concurrence for transfer has been given by the Commanding General, Brooke Army Medical Center, transfer may be made as an emergency case, without prior authority from the Armed Services Medical Regulating Office. The Armed Services Medical Regulating Office will be advised of action taken by the transferring hospital in all such cases. A special research team at the Surgical Research Unit, Brooke Army Hospital, is available for dispatch to any hospital in the United States to aid in the early treatment of burn cases, and to advise hospital commanders on the trans-

portability of such cases. To obtain this team, hospital commanders should telephone or telegraph the Commanding General, Brooke Army Medical Center, Attention: Director, Surgical Research Unit. In order to provide for proper selection of cases for research and to make available the aid of highly qualified personnel of the research group, the Director of the Surgical Research Unit should be advised of the admission to any Zone of Interior Army hospital of all severe burn cases at the earliest practicable date. The importance of early notification cannot be emphasized too strongly.

3) Each case will be considered on an individual basis, with the welfare of the patient being the basic consideration as determined by sound professional judgment.

News and Notes

Scientists in the News

James G. Baxter has been appointed assistant director of research in Eastman Kodak Company's Distillation Products Industries Division. Dr. Baxter has been with Kodak since 1934, and in the 1940s he and his assistants prepared the first pure crystals of vitamins E and A.

David D. Bonnet has been appointed associate research entomologist, Department of Infectious Diseases, School of Medicine, UCLA, where he will be in charge of the Mosquito Control Program of the Pacific Tropic Diseases Project directed by John F. Kessel. Dr. Bonnet was formerly medical entomologist, Department of Health, Territory of Hawaii.

Dean A. Clark has been appointed clinical professor of preventive medicine at Harvard Medical School, where he will instruct students in preventive medicine, and in the social aspects of medicine and medical economics. Dr. Clark will also continue as general director of the Massachusetts General Hospital.

Charles S. Davidson has been appointed associate physician in the Thorndike Memorial Laboratory, Boston City Hospital, and associate professor of medicine, Harvard University. Dr. Davidson's research has centered on nutrition and diseases of the blood and liver, and he has been on the staffs of the university and hospital since 1941.

Robert J. Ferlauto, director of the Microbiological Laboratories of Smith, Kline & French, has been named associate director of development. He will be in charge of exploratory and specialty development, taking over the duties of **Robert H. Tully,** now on active duty with the Army Medical Corps.

Rowan Gaither, Jr., has been appointed acting president of the Ford Foundation, and will direct the work of the foundation until the election of a successor to **Paul G. Hoffman,** who has resigned, effective Mar. 1. Mr. Gaither is a San Francisco lawyer, and has been associated with the foundation since 1948.

Charles F. Gregory, orthopedic surgery instructor of Indiana University Medical Center, now on a Korean War hospital ship, has received the Kappa Delta sorority's national award for research aid to crippled children.

Beno Gutenberg, professor of geophysics and director of the Seismological Laboratory, California Institute of Technology, has been selected to receive the William Bowie Medal of the American Geophysical Union, in recognition of his work on earthquakes, seismicity of the earth, geophysics, and the nature of the earth's core.

Nathan O. Kaplan, of Johns Hopkins University, will receive the annual Eli Lilly and Company Award in Biological Chemistry. Dr. Kaplan helped to discover coenzyme A.

James E. LuValle has resigned as research associate at Eastman Kodak Research Laboratories to take a position as project director with Technical Operations, Incorporated, Arlington, Mass. He will direct a project on fundamental photographic theory, under sponsorship of the Chemistry Division of the Air Research and Development Command.

J. Roscoe Miller, president of Northwestern University, and **Tom D. Spies,** chairman of the Department of Nutrition and Metabolism at Northwestern, have been voted honorary degrees by the medical school faculty of the University of Havana. The degrees are in recognition of Northwestern's studies on foods and their relation to health. Others who will receive honorary degrees are **J. R. Killian, Jr., Robert S. Harris,** and **Robert R. Williams.**

Eger V. Murphree, president of the Permanent Council of the World Petroleum Congress, and president of the Standard Oil Development Company, has recently returned to New York from Rapallo, Italy. He had been attending a meeting of the council to discuss the program and to outline the arrangements for the meeting of the Fourth World Petroleum Congress, to be held in Venice, June 1–8, 1955.

Robert Cushman Murphy, Lamont curator of birds, American Museum of Natural History, has been admitted as one of the 25 foreign fellows of the Zoological Society of London.

Bruce M. Pollock has been appointed plant physiologist, Department of Biological Sciences, University of Delaware, to succeed **J. E. Graustein,** who retired Feb. 1. Dr. Pollock was awarded a National Institutes of Health postdoctoral fellowship for 1950–51, and

studied at Copenhagen, continuing his research at Connecticut College before going to Delaware.

Grover F. Powers, professor emeritus of pediatrics at Yale University, has been named chairman of a national board, sponsored by the National Association for Retarded Children, to direct research on mental retardation.

Edwin Roy Rathbun, Jr., has joined the staff of Radiation Counter Laboratories, Inc., as director of physical research. Formerly with Nuclear Instrument and Chemical Corporation, Mr. Rathbun has worked on design and development of radiation detectors and associated electronic systems for special purposes.

Richard D. Robinson was the third American Universities Field Staff representative to visit California Institute of Technology this year. Mr. Robinson lectured and led discussions and seminars on his observations in Turkey, where he has studied political, economic, and social conditions.

Raphael Rosen, of Standard Oil Development Company, has retired after 26 years of service. He joined the Development Department of Standard Oil in 1923, and the Development Company in 1927, when it was formed.

Richard H. Shryock, director, Institute of the History of Medicine, Johns Hopkins University, will give the tenth D. J. Davis Lecture on Medical History at the University of Illinois College of Medicine, Chicago, on May 6. The lecture will be entitled "Changing Concepts in American Medicine over Three Centuries."

Herman T. Spieth, entomologist, has been named professor of zoology and chairman of the Life Sciences Division of the University of California's new College of Letters and Science at Riverside. He has been a member of the biology staff at City College, New York, for 20 years.

Phrixos J. Theodorides, formerly consultant in aerophysics, U. S. Naval Ordnance Laboratory, White Oak, Md., has been appointed research professor of the Institute for Fluid Dynamics and Applied Mathematics, University of Maryland.

John B. Whitney, Jr., has resumed his post as associate professor of botany, Clemson Agricultural College, after serving for a year as a research participant at the University of Tennessee-Atomic Energy Commission Agricultural Research Program at Oak Ridge.

Louis L. Williams, Jr., chief of the Division of International Health, USPHS, retired on Jan. 31. Dr. Williams, a specialist in malaria control research for 26 years, long directed Public Health Service malaria investigations.

David G. Wilson has been appointed to the staff of the Biochemistry Department of the Connecticut Agricultural Experiment Station.

Education

The **University of Chicago** has received an anonymous gift of $300,000 for the endowment of a distinguished service professorship honoring Robert M. Hutchins, former chancellor. The anthropologist Robert Redfield has been appointed to the professorship.

Duke University will give a course in acarology June 10–29 under the direction of G. W. Wharton, Department of Zoology. Three visiting lecturers—Edward W. Baker, U. S. Department of Agriculture, A. Earl Pritchard, University of California, and R. W. Strandtmann, Texas Technological College—will participate.

George Washington University has received a grant from the U. S. Air Force for research into the effects of different diets on the body in zero temperatures. The work will be under the direction of Carleton R. Treadwell, professor of biochemistry.

Indiana University, under a contract with the Office of Ordnance Research, has appointed E. Titt, of the University of Texas, as visiting associate professor at the Graduate Institute for Applied Mathematics. J. Eriksen, of the Naval Research Laboratory, has been made a research associate for the current semester, and A. Van Tuyl, of the Naval Ordnance Laboratory, and Ion Carstoiu, of Johns Hopkins, have been made research associates for the second semester.

The **University of Maryland** Institute for Fluid Dynamics and Applied Mathematics will present the following public lectures: Mar. 18, Problems in Conformal Mapping, Lars V. Ahlfors; Apr. 8–9, Mechanics, H. G. Kuessner, of the Max Planck Institut for Stroemungsforschung; May 6–7, Behavior of Solutions of Partial Differential Equations, Fritz John.

New York University has established a new doctoral program in the field of remedial reading. Nila Banton Smith will be in charge of the courses, which are designed primarily for teachers, clinicians, and psychologists.

Polytechnic Institute of Brooklyn, under the sponsorship of Sylvania Electric Products Inc., will present a series of lectures by H. K. Henisch, of the University of Reading, on the theory of semiconductors. Two-hour lectures will be given from 8:00 to 10:00 P.M., Apr. 23 and 30, and May 7 and 14.

The **Tissue Culture Association** is sponsoring a course in the principles and techniques of cell and tissue culture, under the direction of John H. Hanks, in the laboratories of the Mary Imogene Bassett Hospital, Cooperstown, N. Y., July 8–30. The course is designed specifically for M.D.s or Ph.D.s who plan to use such techniques in their research or teaching. Application forms, returnable not later than *Apr. 15,* may be obtained from Mary S. Parshley, College of Physicians and Surgeons, 630 W. 168th St., New York 32.

The **University of Toledo** has acquired the Pittsburgh Plate Glass Company as a supporting member of its Graduate Institute in Silicate Chemistry and Related Sciences. Other participants in the institute, which was organized last summer, are Libbey-Owens-Ford Glass Company, Owens-Corning Fiberglas Corp., and Owens-Illinois Glass Company. Wilhelm Eitel, founder of the Kaiser Wilhelm Institute of Silicate Research, is director of the institute.

Because of staff shortages in the Department of Electrical Engineering at the **University of Wisconsin**, John R. Price and Leslie E. A. Kelso, both of whom retired in June 1950, are serving on special teaching contracts during the current semester. William H. Peterson, a third emeritus professor, will continue his research in biochemistry, which is supported by Merck and Squibb.

The semiannual **X-Ray Diffraction School** of the North American Philips Company, Mount Vernon, N. Y., to be held Apr. 20–24, will offer the following speakers: Apr. 20–21, I. Fankuchen; Apr. 22, William Parrish; Apr. 23, Herbert Friedman; Apr. 24, Ray Pepinsky, Martin Buerger, Richard Bear, and others. Registration, for which there is no fee, is limited to 100, with applications filled in order of receipt.

Grants and Fellowhips

The **Arthritis and Rheumatism Foundation** has awarded ten new grants and renewed six (totaling $77,300), for basic research involving the connective tissues. Baruch S. Blumberg, William C. Robbins, Thomas G. Kantor, Robert Rowen, Harold B. Houser, Alvin Volkman, Vaughn W. Westermeyer, Charles W. Denko, Allan St. J. Nixon, and David Platt are the recipients of new grants.

Bethlehem Fabricators, Inc., has endowed a scholarship at Lehigh University in honor of R. P. Hutchinson, chairman of its board. The first award will be made at the end of the 1953–54 academic year. Lehigh has also established a new scholarship for students in mining engineering, through a bequest from the late Esther R. Fuller Warwick, given in memory of her father, **John T. Fuller.**

A **Joseph De La Roche D'Aillon** scholarship has been established at St. Bonaventure (N. Y.) University by John P. Herrick, oil producer and writer, of Olean, N. Y. The scholarship honors the Franciscan missionary who, three centuries ago, became the first white man to report the presence of oil in North America.

Duke University Marine Laboratory is offering ten graduate scholarships, covering tuition, board, and room, for courses in marine ecology, oceanography, coastal aquatic vegetation, marine invertebrate zoology, or for research in marine botany and zoology. Application and transcript should be sent to C. G. Bookhout, Department of Zoology, Duke University.

The **V. N. Ipatieff Research Professorship in Organic Chemistry** has been endowed at Northwestern University, by Universal Oil Products Company in honor of the company's late director of chemical research.

The **Frank B. Jewett Postdoctoral Fellowships** for 1953–54 have been won by Marshall Fixman, of MIT; Henry Helson, of Yale; Roger Gerhard Newton, of Harvard; Richard Scott Pierce, of Yale; and Daniel Burrill Ray, of Cornell. Dr. Helson was among the award winners last year.

The **Henry J. Kaiser Family Foundation** has given $50,000 to the University of California to provide for grants-in-aid to medical students in the Schools of Medicine in San Francisco and Los Angeles, and for graduate nurses in San Francisco, Berkeley, and Los Angeles. The foundation suggested that the total, together with any interest, be expended within the next three to five years.

The **National Foundation for Infantile Paralysis** has approved research and professional education projects totaling $2,586,271 at 32 medical schools, hospitals, research institutions, and educational organizations. Among the largest grants was $104,486 to the University of Toronto–Connaught Medical Research Laboratories, for work under the direction of Andrew J. Rhodes. Other large sums went to the University of Pittsburgh ($236,472, for Jonas E. Salk); Mount Sinai Hospital, New York ($185,458, for Gregory Shwartzman); and the University of Cincinnati ($114,280, for Albert B. Sabin).

The **Rapkine French Scientist Fund** has undertaken to assist an American biochemical geneticist to travel to the ninth International Congress of Genetics, which will be held in Bellagio, Italy, Aug. 24–31. The foundation was created in memory of the late Louis Rapkine to aid scientists of all nations who work in fields related to Rapkine's. Francis J. Ryan, Department of Zoology, Columbia University, is chairman of the Genetics Society Travel Committee.

In the Laboratories

American Bio-Chemical Laboratories, Inc., has appointed S. L. Goldheim director of its laboratory at 1612 Harford Ave., Baltimore. Dr. Goldheim, who has been doing research in the chemical and fermentation industries, will consolidate his own office and laboratory with that of ABC.

The **Atomic Energy Commission** will build an explosives processing and assembly plant on the Spoon River in Fulton County, near Macomb, Ill. Construction will begin this spring. The new plant will not manufacture radioactive material.

Ethyl Corporation has appointed Richard C. Back, an entomologist, to the product development staff for research on agricultural chemicals. John D. Bartleson, a specialist in hydrocarbon synthesis and lubricating oil additives, has joined the Automotive Products Division in Detroit.

Foundation Laboratories, Inc., has been formed to develop and distribute the steroid hormone products of Syntex, S. A. John R. Mote, of Smith, Kline & French, has been appointed medical director, and Jack Reed, of Chemical Specialties Co., will head the Division of Veterinary Medicine and Animal Husbandry.

Hercules Powder Company will lend Henry Reeves, Jr., to the Hercules Powder Company, Ltd., in England, for two years. Mr. Reeves is assistant to the director of operations for the Paper Makers Chemical Department.

Eli Lilly and Company have appointed Raymond E. Crandall head of the Antibiotics Fermentation Department to succeed J. J. Stefaniak, who has been named assistant director of Lilly's new Tippecanoe Laboratories. Recent additions to the Lilly staff include Lawrence E. Barron, Marguerite R. Clark, William L. Garbrecht, and Walter E. Wright.

Los Alamos Scientific Laboratory has added the following physicists to its staff: John F. Barnes, Joe Bergstein, Maxine S. Goad, and Alfred W. Solbrig. Bruce J. Dropesky, chemist, has joined the Test Division.

Meetings and Elections

American Chemical Society division elections include the following: Division of Analytical Chemistry: Herbert A. Laitinen, chairman, and G. Frederick Smith, chairman-elect; Division of History of Chemistry: Virginia Bartow, chairman (re-election); Biological Chemistry: Richard H. Barnes, chairman, and Esmond E. Snell, vice-chairman (re-elections); Carbohydrate Chemistry: Thomas R. Gillett, chairman, and Nelson K. Richtmyer, chairman-elect; Gas and Fuel Chemistry: George D. Creelman, chairman, and Corliss R. Kinney, chairman-elect; Physical and Inorganic Chemistry: F. A. Long, chairman, and Joseph W. Kennedy, chairman-elect.

The **Gerontological Society, Inc.,** will hold its sixth annual scientific session in San Francisco, Aug. 26–28, under the chairmanship of Edward L. Bortz, of Philadelphia. Harold Jones, Department of Psychology, University of California, Berkeley, is chairman of local arrangements.

The third **International Biometric Conference** will be held at Bellagio, on Lake Como in northern Italy, Sept. 1–5, immediately following the **International Genetics Congress** in Bellagio, Aug. 24–31, and preceding the **International Microbiological Congress** in Rome, Sept. 6–12. Members living in the U. S. should send their registration cards and remittances at once to the Biometric Society, Box 1106, New Haven 4, Conn. Others should apply directly to the executive secretary of the conference, L. L. Cavalli-Sforza, Istituto Sieroterapico Milanese, Via Darwin 20, Milan. Full information concerning each contributed paper or exhibit should reach the secretary before *Apr. 30*, and should include the title, a summary in English of 200 words or less, and other pertinent details.

The **XIVth International Congress of Zoology** will be held in Copenhagen Aug. 5–12. R. Spärck is president of the congress, and Anton Fr. Bruun, Zoologisk Museum, Universitetet, Krystalgade 27, Copenhagen, is secretary general. The preliminary plan is to have three general meetings and several sections, with two excursions to West and East Jutland after the congress, and possibly one to study the marine fauna of the deeper part of the Skagerrak.

The **Modern Language Association** has elected Hayward Keniston, of Duke University, president for 1953; Adolf Benson, of Yale, and Thomas Clark Pollock, of New York University, were elected vice presidents. Juan R. Castellano, of Duke, was elected vice president of the **American Association of Teachers of Spanish and Portuguese,** an MLA affiliate.

The **National Vitamin Foundation** will hold its annual meeting Mar. 4 at the Biltmore Hotel, New York. Among participants in the scientific session will be Karl E. Mason, E. W. McHenry, B. M. Kagan, W. F. Alexander, M. K. Horwitt, William J. Darby, Nevin S. Scrimshaw, Roberto Funaro, and W. T. Thompkins.

The annual meeting of the **Radiation Research Society** will be held at the State University of Iowa, June 22–24. E. S. G. Barron, Edwin J. Hart, Warren Garrison, J. L. Magee, and A. O. Allen will participate in a symposium on "The Effects of Radiation on Aqueous Solutions," and Ugo Fano, Burton J. Moyer, G. Failla, L. D. Marinelli, and Payne S. Harris will discuss "Physical Measurements for Radiobiology." L. H. Gray, of the Hammersmith Hospital, London, will give a special lecture on June 23. For full information, address A. Edelmann, Biology Department, Brookhaven National Laboratory, Upton, N. Y.

A Symposium on the Future and Limitations of Physiology will be held under the auspices of the International Union of Physiological Sciences in Montreal during the meetings of the International Physiological Congress, Aug. 31–Sept. 4. The symposium is being underwritten by a grant from the International Council of Scientific Unions, and the first meeting will be a public one.

A Symposium on Recent Advances in the Study of Venereal Diseases will be held Apr. 30–May 1 in Washington, D. C., under the auspices of the Experimental Therapeutics Study Section, National Institutes of Health. A preliminary program will be published in the March number of the *American Journal of Syphilis, Gonorrhea, and Venereal Diseases.* For further information, address Frederick W. Appel, at NIH, Bethesda 14, Md., or John C. Hume, American Venereal Disease Association, 615 N. Wolfe St., Baltimore 5.

A Symposium on Stress, cosponsored by the Army Medical Service Graduate School and the National

Research Council Division of Medical Sciences, will be held in Washington, D. C., Mar. 16–18. Among the speakers will be John C. Whitehorn, Wallace O. Fenn, Hudson Hoagland, and Henry Brosin. Visitors from abroad will include Ulf Svente von Euler, head of the Physiology Department of the Karolinska Institutet, Stockholm, and Ludwig Guttmann, director of the Spinal Injuries Centre, Aylesbury, England.

The **Texas Academy of Science,** at its annual meeting held on the campus of Texas Christian University, elected D. B. Calvin president and Joseph P. Harris vice president. Gladys H. Baird was re-elected secretary-treasurer. More than 700 persons registered for the meeting, over half of which were members of the Collegiate and Junior academies. The Collegiate Academy now has 29 chapters and about 400 individual members. Approximately 30 papers were presented by students; many of the papers will be published in *Tasca,* the quarterly journal of the academy.

Miscellaneous

The **American Forestry Association** is again offering a ten-day horseback trip (May 20–30) in the Great Smoky Mountains. Headquarters will be the Cataloochee Ranch, on Fie Top Mountain, near Waynesville, N. C. Cost of the trip is $175, from Asheville, where riders will be met and transported to the ranch, and returned on the morning of May 30. For reservations and full details, write to Dorothy Dixon, American Forestry Association, 919 17th St., N.W., Washington 6, D. C.

A field trip sponsored by the **American Museum of Natural History** will take Harry Tschopik, Jr., assistant curator of ethnology, and Rául de los Ríos, of Peru, to the tributaries of the upper Amazon. Dr. Tschopik will make a socioanthropological study of the Indians, and Mr. de los Ríos will make a collection of the food, weapons, clothing and artifacts of these people.

The **American Society for Metals** has established a Foundation for Education and Research. Approximately $30,000 a year will be available for the advancement and dissemination of scientific knowledge, particularly with respect to the technology of metals, for the use and benefit of the public at large, whether through education and research carried on by the foundation or through the support of educational and research activities in established institutions.

The **California Foundation for Biochemical Research,** 3408 Fowler St., Los Angeles 63, has available 10 mg samples of thymidylic acid, an important component of living cells, which will be distributed free to qualified researchers upon request.

The **Capricorn Expedition,** conducted by the Scripps Institution of Oceanography, and sponsored by the Office of Naval Research, will have as a primary objective the study of the geology and geophysics of the South Pacific. Two ships, the R/V *Horizon,* of the University of California's research fleet, and the R/V *Spencer F. Baird,* owned by the Navy and operated by Scripps, will take the expedition to the Fiji Islands, over the Tonga Deep, through the Society and Marquesas islands, across the Easter Island swell and the Albatross Plateau, and finally north to San Diego. Roger Revelle, director of Scripps, and member of the Pacific Science Association Standing Committee on Pacific Oceanography, will be scientific leader of the expedition. Like the Northern Holiday Expedition of 1951 and the Shellback Expedition of 1952, Capricorn has been planned to enlarge knowledge of a little-studied oceanic area.

Among reports recently issued by the United Kingdom's **Department of Scientific and Industrial Research** are: *Pest Infestation Research 1951,* which devotes noteworthy attention to blowflies, cheese mites, special sources of infestation, and problems of control by means of insecticides and fumigants; and *Radio Research 1951,* which stresses recent research on the properties and electrical applications of germanium, radio wave propagation especially in the ionosphere, and a project involving a geographic survey of high-frequency radio noise levels that interfere with long-distance radio communication. Both reports are available at the British Information Services, Rockefeller Center, New York.

Recent visitors from abroad at the **Eastern Regional Research Laboratory,** Philadelphia, were Karl Pedersen, Copenhagen Pectin Factory; P. Hedemann, Kolding-Egnens Mosteri, Kolding, Denmark; and Marry H. Lykaas, Joint Establishment for Nuclear Energy Research of Norway, Lillestroem.

The **Fund for the Republic** has been established by the Ford Foundation, to work for the elimination of restrictions on freedom of thought, inquiry, and expression in the U. S. A group of distinguished men and women has been asked to form an independent organization and to outline a program. If the group's work proves satisfactory, a substantial grant will be made to enable it to carry on its work over a period of years.

The **Future Scientists of America Foundation** will give 104 awards to students, grades 7–12, and 20 to teachers, for projects and activities relating to science and mathematics, in its second annual program of science achievement awards. The foundation is sponsored by the American Society for Metals, and the program is administered by the National Science Teachers Association. Rules and information may be obtained from the foundation, 1201 16th St., N.W., Washington, D. C.; reports of projects must be sent to regional chairmen by *May 31.*

A combined index for Vols. 1–58, 1903–51, of the ***Proceedings of the American Society for Horticultural Science,*** prepared by H. B. Tukey and M. S. Barrett, is now available from the W. F. Humphrey Press, Geneva, N. Y., at $4.00.

Technical Papers

Intermediate Compounds Formed when Horseradish Peroxidase Reacts with Potassium Molybdicyanide and Potassium Chloriridate

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The titration of compound II of peroxidase with reducing agents[1(a)] showed it to be one equivalent above the ferric state of the enzyme and thus a compound containing iron with an effective oxidation number + 4. This leads to a new mechanism (*1*) for peroxidase action based on one-equivalent reduction steps:

$$\text{Per} + H_2O_2 \longrightarrow \text{compound I} \xrightarrow[\text{reduction}]{\text{one equiv}}$$

$$\text{compound II} \xrightarrow[\text{reduction}]{\text{one equiv}} \text{Per} \quad . \quad . \quad . \quad a$$

The reduction of compound I to compound II has subsequently been confirmed in a direct titration by Chance (*2*). Accordingly, compound I could be a *complex* containing H_2O_2 or its anion O_2H^-, or a *compound* of iron with an effective oxidation number + 5. Other oxidizing agents, HOCl, HOBr, $NaClO_2$, ClO_2, etc., were found to replace H_2O_2 in a typical peroxidase reaction, the oxidation of guaiacol to tetraguaiacol.[1(b)] These oxidizing agents give intermediate compounds like H_2O_2: with cytochrome-*c* peroxidase an intermediate indistinguishable from compound II is formed exclusively as the stable intermediate, whereas with horseradish peroxidase the resulting absorption spectra are typical of a mixture of compounds I and II. The redox properties of compound II and the fact that the spectra obtained with the various oxidizing agents are identical within experimental error suggest a common structure in all systems. These oxidizing agents, however, possess two or more oxidizing equivalents, and the underlying chemical changes are thereby obscured. The recent discovery by George and Irvine (*3*) that simple single electron transfer oxidizing agents like potassium chloriridate and molybdicyanide can oxidize metmyoglobin to its effective quadrivalent iron compound (*4*), previously described as a hydrogen peroxide complex (*5*), thus called for similar experiments with peroxidase.

[1] P. George. *Nature*, **169**, 612 (1952), which contains a summary of four papers recently published: (*a*) The chemical nature of the secondary hydrogen peroxide compound formed by cytochrome-*c* peroxidase and horseradish peroxidase. (*b*) The formation of intermediate compounds when horseradish peroxidase and cytochrome-*c* peroxidase react with strong oxidizing agents. (*c*) Experiments on the third intermediate compound formed when hydrogen peroxide reacts with horseradish peroxidase. (*d*) A comparison of the effect of strong oxidizing agents on horseradish peroxidase, haemin, and tetrasulfonated copper phthalocyanine.

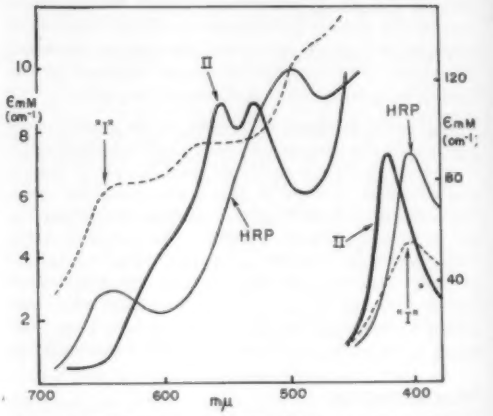

FIG. 1. Spectra of the first intermediate, "I," and compound II formed by the action of potassium chloriridate on horseradish peroxidase. Soret region: 1.5 μM-enzyme, 16.4 μM-K_2IrCl_6 at pH 10.2; visible region: 4.5 μM-enzyme, 34.5 μM-K_2IrCl_6 at pH 9.2. (Spectrum of peroxidase denoted by HRP.)

A sample of horseradish peroxidase prepared by Keilin and Hartree's method was used (*5*). With 1.5 μM enzyme and about 35 μM potassium molybdicyanide, full formation of an intermediate identical to the previous compound II was obtained at pH 11.0; reduction by potassium ferrocyanide regenerated the enzyme completely. At pH 9.2 only 40% conversion occurred. The presence of cyanide ions in some molybdicyanide solutions was occasionally observed by the production of the peroxidase-cyanide complex instead of compound II and its consequent resistance to reduction by ferrocyanide.

Using the same enzyme concentration and a five- to tenfold molar excess of potassium chloriridate, the reaction follows a different path. At pH 9.2–11.6 an intermediate is formed first characterized by low absorption in the Soret region and strong absorption in the visible extending to 650 mμ. This then changes spontaneously into a second intermediate having the spectroscopic characteristics of compound II. Both spectra are shown in Fig. 1. The first intermediate is apparently stable while excess potassium chloriridate is still present, for its lifetime is longer the higher the initial iridate/enzyme ratio. The addition of one equivalent of potassium ferrocyanide converts the first intermediate very rapidly into compound II before the reduction of compound II to the ferric state of the enzyme, which is recovered fully on the addition of more ferrocyanide. When potassium chloriridate is added to compound II, formed either from the spontaneous reaction of the first intermediate or from hydrogen peroxide itself, the first intermediate again appears and then reverts to compound II again. Excess hydrogen peroxide converts the first intermediate

into compound II or compound III if the concentration is high enough—i.e., greater than 1.0 mM, as was found in the hydrogen peroxide system alone (*5*).[1(e)]

These results cannot be due to the initial formation of peroxides through the attack of the oxidizing agents on reducible groups present, for peroxides themselves do not react in an identical way. They reveal further the similarity that exists between compound II of peroxidase and the metmyoglobin intermediate, for both can be formed using single electron transfer oxidizing agents.

Compared with metmyoglobin, the appearance of the first intermediate when potassium chloriridate is used presents an additional feature of great importance, because its chemical properties parallel those of compound I in the H_2O_2 or alkyl hydroperoxide systems (*6*). It is reduced to compound II by ferrocyanide in accord with the above reaction scheme *a*, and appears again when compound II is oxidized by potassium chloriridate. It differs from compound I in the peroxide systems by its far greater stability and certain details of its absorption spectrum (*7*). Although it has a Soret band of similar low intensity, the absorption is somewhat greater below 405 mμ. There can be little doubt that it is a compound of the same chemical type, and in conjunction with the spectroscopic indications that an intermediate of low Soret band intensity is also formed with the other oxidizing agents, an enzyme-substrate-complex structure for compound I, Per-H_2O_2 or Per-OOH, appears less satisfactory compared with more general structures in which the iron has the effective oxidation number + 5.

Further experiments showed that intermediates are only produced using potassium chloriridate in solutions of pH greater than 7.0, and that at pH 11.6, where the peroxidase is present in its alkaline form, the very rapid production of compound II via the first intermediate still occurs. This behavior is in contrast to the action of peroxides where reaction with the acid form of peroxidase is pH-independent and the alkaline form does not react (*6*). This suggests that the intermediates can be produced by electron transfer from the acid or alkaline form of peroxidase with chloriridate, whereas the peroxide reactions involve hydrogen atom transfer which is unfavored with the alkaline form. Since the redox potential of the chloriridate ion is independent of pH, these observations also confirm the participation of H in the redox reactions of the intermediates for which there are clear indications in the previous experiments.[1(e)]

No evidence could be found for the reduction of the first intermediate by the enzyme itself in the reaction

First intermediate + enzyme → 2 compound II . . . *b*
(formally: $Fe^{5+} + Fe^{2+} \rightarrow 2\ Fe^{4+}$).

An over-all reaction of this type is undoubtedly favored in the peroxide systems,[1(e)] for at low values of the H_2O_2/enzyme ratio the formation of compound II approximates to the stoichiometry

1 mol $H_2O_2 \equiv$ 2 mol compound II.

The absence of reaction *b* can be regarded as a reason for the appearance of the first intermediate as a stable state. In terms of free energy, reaction *b* will be favored if the redox potential $E^\circ_{5+/4+} > E^\circ_{4+/3+}$. Compound formation by the + 5 state in the chloriridate system would lower $E^\circ_{5+/4+}$, and the stability of the first intermediate would follow on the reversal of this relationship when $E^\circ_{5+/4+} < E^\circ_{4+/3+}$.

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Centrifugation in Field-aligning Capsules: Preparative Microcentrifugation[1]

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Preparative high-speed centrifugation is commonly employed for fractionating and concentrating many biological entities of small size. Some of these materials, notably viruses, may be obtained as highly active agents even in extremely small quantities. This circumstance makes it appear that microcentrifugation methods can be advantageously applied to those cases where only fractional milliliter samples of source material are available—quantities too small to be handled with the usual preparative centrifugation methods. A microtechnique for improving the specific activity of minute amounts of sedimentable materials serves also as a useful adjunct to microchemical and microphysical assay methods. Severe difficulties arise in standard centrifugation procedures when attempts are made to secure successive depositions of a small amount of material in round-bottom plastic tubes of the size commonly employed in angle rotors, since excessive losses of recoverable material occur owing to "shelving" and back-diffusion from the thinly spread deposits. Capillary centrifugation in swinging-cup rotors has previously been employed (*1–3*), but the largest values of centrifugal acceleration obtainable with such rotors are too small to pellet most viruses and macromolecules.

A method that might be described as "field-aligning capsule centrifugation" has been employed in this laboratory for the subfractionation of very small pellets obtained from 10-ml volumes of clarified supernatant fluid of virus-infected tissue cultures and controls. Pellets obtained initially by use of conventional equipment are resuspended in 0.01–0.1 ml of diluent

[1] This research has been supported in large part by a grant from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

fluid, and centrifugation of this resuspension in small glass capsules compacts the particulate components into tips tapering to microscopic dimensions. The capsules are so prepared for centrifugation as to include an air bubble which serves initially to float the capsules vertically in standard plastic centrifuge tubes filled with a liquid adjusted to a suitable density. During the centrifugation cycle the long axis of the capsule is constantly aligned with the vector resultant of the three forces acting upon it: gravitation, and the radial and tangential forces resulting from the rotational motion. Effectively, the alignment of the capsule is as perfect as though it were a cup swinging on a truly universal bearing.

Two stages of preparation are required prior to centrifugation: (*a*) encapsulation of the material to be centrifuged and (*b*) flotation of the filled capsule. The stepwise procedure for encapsulation is illustrated in Fig. 1. The capsule is first prepared as a micropipette, with capillary stems drawn from each end of the body, which remains as a segment of thin walled glass or quartz tubing. Suitable thin-wall glass stock may be prepared by using glass apparatus-weight tubing of 15-mm diameter, and by drawing out a 2- or 3-cm section of it to a length of about 20 cm. In drawing out the capillary stems one must take care not to produce too thin a capsule wall near its ends. For centrifugation in fields above approximately 40,000 *g* the capsule walls must be of uniform thickness and circular in cross sections. Quartz capsules are notably stronger than glass ones and can be centrifuged at over 100,000 *g* without breakage. They can be readily prepared from thin-walled quartz tubing by use of a fine oxygen-gas flame. It has been found advantageous to make a number of pipettes with varying capacities to facilitate matching the capsule volume with the sample volume.

Material for encapsulation is first clarified of gross particulate material and then siphoned into one of the capsules of adequate capacity to contain the sample and also to enclose a bubble of air, as indicated

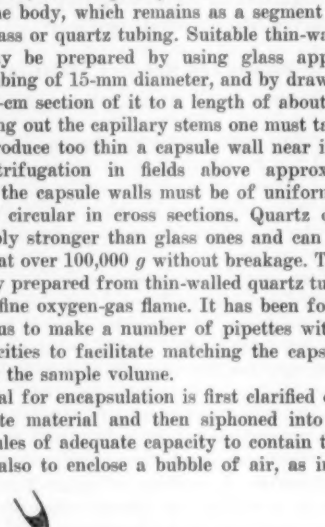

FIG. 1. Steps in the preparation of glass capsules for microcentrifugation. *A*, filling the open-ended capsule; *B*, sealing the solvent-containing end; *C*, sealing an air bubble into the other end; *D*, breaking elongated end after transposing bubble by momentary centrifugation; *E*, final sealing of air-bubble end.

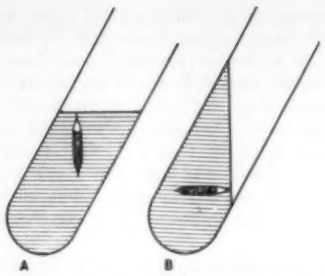

FIG. 2. Capsule floating in partially filled centrifuge tube. *A*, rotor at rest; *B*, rotor at speed. For high-speed centrifugation the tube must be filled by floating mineral oil over the salt solution shown.

in Fig. 1 *A* and *B*. The optimum size of the enclosed bubble clearly depends upon the average density of the capsule compared with the medium in which the capsule is to float, but a considerable latitude in bubble size may be compensated for by variation in the density of the flotation medium. A minute amount of clean solvent sufficient to displace the previously admitted solution from the stem into the body of the capsule is admitted, and the entrance tip of the long stem is sealed quickly in a fine, hot flame. The open capillary stem is sealed off close to the body by first drawing it out at the point of sealing to a thread, followed by fusing (Fig. 1 *C*). The fused end is immediately quenched with a drop of water to prevent warming the capsule contents excessively. If the capsule is of short body length it is ready for use at this stage, since the material sedimenting during centrifugation will collect in the elongated tip through which the sample was admitted. But if capsules are to be made of the greatest possible capacity, further preparation is required to allow them to fit closely across the plastic tubes of the angle rotor employed. The bubble is transposed to the opposite end of the capsule by a momentary centrifugation at low speed, the tip-seal of the remaining capillary stem is severed (Fig. 1 *D*), and the stem removed in the manner described previously. The completed capsule is shown in Fig. 1 *E*. Two fractions may be obtained from one encapsulation in the following manner: the capsule is first used at the stage shown in Fig. 1 *C* in a rotor provided with plastic tubes of diameter sufficient to permit horizontal orientation of the capsule with stem attached; after centrifugation the stem and its contents are removed; the capsule is then resealed for a subsequent sedimentation in a smaller rotor capable of higher values of centrifugal field.

The suspension of the capsule is illustrated in Fig. 2. A plastic centrifuge tube is half filled with a salt solution adjusted to a density such that the capsule is supported in a vertical position with the upper tip slightly above the surface of the salt solution. During centrifugation the capsule must be free to swing through 90° of arc in a plane containing the rotor axis. The greatest possible length of a capsule floating

freely in a tube of an angle rotor is determined by the diameter of the centrifuge tube containing the capsule and the angle at which the tube is held (the "rotor angle"). A 45° rotor angle permits the greatest capsule length for a given tube diameter.

When high centrifugal fields ($> 20{,}000\ g$) are employed the possible collapse of the plastic centrifuge tubes is prevented by filling them completely, with mineral oil layered above the aqueous level. After centrifugation the capsule may conveniently be removed from the centrifuge tube by first lowering over it an open end of a closely fitting section of glass tubing. When the tube is withdrawn, the capsule and some salt solution come with the tube. The capsule is rinsed and blotted dry and then enveloped in a piece of transparent Scotch tape. It is then scored through the tape at a position slightly above the meniscus, and broken apart. Supernatant fluid can be readily removed by capillary siphoning, and the sediment resuspended in fresh solvent with the aid of glass probes.

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Establishing Long-Term Cultures of Mammalian Normal, Solid Tumor, and Ascites Tumor Cells on Glass

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By embedding his tissue fragments in a clot of lymph Harrison (*1*) was the first to succeed in growing or maintaining animal cells *in vitro*. Shortly thereafter, Burrows (*2*) suggested the use of a plasma clot as the solid phase of tissue culture media, and this technique has become very widely adopted by tissue culture workers.

The practice of embedding the tissue to be cultured in a clot, although originally the difference between success and failure, has greatly restricted the dimensions of tissue cultures and has placed serious limitations on experimental applications, especially in the field of metabolism. To cope with the problem, various investigators have tried replacing the plasma clot with simpler materials or else have sought means for separating the cells from the plasma. Outstanding among these have been Evans and Earle and their associates, who introduced perforated cellophane (*3*) as a solid substrate for cell growth, and later found that certain lines of cells, after growing on the cellophane, could be induced to grow directly on the glass surface of the culture vessel. Interest in obtaining animal cell cultures of the type described by Earle (strain L) led to the development of the techniques presented in this paper for establishing and maintaining the growth of several cell strains directly on glass.

A slight modification in the customary roller tube culture technique was employed for the original cultures. It is the general practice to coat the entire inner surface of the roller tube with clotted plasma; this was modified by using only a strip of plasma, about ¼ in. wide, streaked down one side of the tube with a capillary pipette. Three explants approximately $6 \times 2 \times 1$ mm were seated in this strip, and coagulation was induced by the addition of a drop of 50% chick embryo extract. One ml of medium of the following composition was added to each tube: 10% chick embryo extract (final concentration), 50% Earle's balanced salt solution, and 40% horse serum. The tubes were incubated at 37° C on a conventional roller tube apparatus. Fluid changes were made 3 times weekly unless the condition of the cultures indicated that a departure from this routine was desirable. Good growth was noted generally within 48 hr in the plasma around the explants, and later, as the area of growth increased, the cells were observed to migrate out of the plasma onto the glass. After about 2 months' incubation, growth on the glass usually extended completely around the tube, covering all, or nearly all, the surface of the lower half. Covering of the area was not always accomplished by a steadily advancing sheet of cells, but often through random scattering. Apparently cells or cell clumps became detached from areas of dense growth, floated free, and resettled in other open areas. These islets then started new growth centers and thus speeded up total population of the available surface. After this amount of growth had occurred, nearly all evidence of the original explants had disappeared and most of the narrow strip of plasma had been lysed or otherwise lost. Thus a dense culture of cells growing directly on glass had been achieved.

After obtaining growth of the cells on glass, the next and more perplexing problem was that of subculture by the scraping and pipetting technique. Most experiments fail at this point, apparently because the cells do not survive such harsh physical treatment or change of environment. To facilitate this step, two recommendations found in the literature of Sanford and Earle and their associates were followed. The first of these involved the use of "conditioned medium," as described for single cell experiments (*4*); i.e., the cells were scraped into the old medium, and this suspension was then transferred to the new vessel with no further addition of medium. In most instances the pH of this medium was quite low; hence adjustment was made to approximately pH 7.6 by adding a few drops of sodium bicarbonate.

The second step was based on Earle's (*5*) finding that a heavier cell suspension favors transplantability. In order to utilize this principle, the first subcultures were made to vessels with a smaller surface area than that of the parent culture flask, thereby concentrating

the cell population. The growth in the roller tube covered an area of at least 3000 mm², which was greater than three times the 950 mm² surface of Carrel D3.5 flasks (as supplied by Otto Hopf, Upper Black Eddy, Pa.) employed for the first daughter cultures. By taking advantage of this difference in the size of the culture areas, a dense population of cells was transferred from the roller tubes to the Carrel flasks, thereby favoring their survival and continued growth. After various intervals, ranging from 2 weeks to several months, the growth in the Carrel D3.5 flasks was scraped off and transferred to Carrel D5.0 flasks, which have a surface area of 1960 mm². Each of the latter cultures in turn was later transferred to two or more D5.0 flasks. Several of the cell strains thus obtained have been successfully transferred from the D5.0 flasks to Earle's large T-60 flasks, each having a floor area of 6000 mm², and have rapidly covered the entire floor area of the flasks. The cultures are being carried in these flasks as stock cultures.

Four cell strains have been established to date, using the above procedure for their isolation. These include the cells of the mouse tumor Sarcoma 180, the Walker rat carcinoma 256, and two apparently different cell types grown from normal embryonic mouse skin. The cells of the Walker carcinoma appear to be epithelial in character, and differ considerably in their morphological and growth properties from the connective tissue cells of the other cultures. Complete descriptions and characteristics of all these cell strains will be published at a later date.

Two additional cell strains have been isolated and established in the type of culture described above. One of these was grown from fragments of a mouse spontaneous tumor and was obtained more through good fortune than planned procedures. In this experiment the explants were embedded in thick plasma clots (approximately 2.0 mm) in Carrel D3.5 flasks. Following 3 months of growth, a hole 1.5–2.0 cm in diameter had developed in the plasma clot of one of the cultures; microscopic examination disclosed that the area of the glass exposed by the lysis of the clot was completely covered with a growth of very healthy cells. While the flask was held firmly to prevent surging of the medium, this area was scraped and the cells so loosened from the glass were drawn up into a capillary pipette along with about 0.5 ml of medium. The cells were transferred to another Carrel D3.5 flask, where they quickly settled onto the glass surface and continued to proliferate, completely covering the floor of the flask within 2½ weeks. This strain of cells is at present over a year old, has been transferred numerous times, still multiplies rapidly, and appears quite healthy in every respect. The designation LLC-M_1 has been applied to the cells and will be used henceforth.

Although it was originally thought that the LLC-M_1 strain was derived from a spontaneous tumor, subsequent histological examination of this growth revealed that it was more likely to be a hypertrophic lymph node. This conclusion was further supported by the fact that the mass occurred in the popliteal region of the leg. There also was considerable doubt that this swelling, or lymph node, had undergone malignant changes, and if such had occurred it was of the lymphatic elements, not of the fibroblastlike cell grown in the cultures. The cultured cells also have failed in several attempts to produce tumors following injection into mice. It is now thought, therefore, that strain LLC-M_1 is a normal adult connective tissue cell grown either out of the stroma of the node or from adjacent subcutaneous tissue.

The remaining strain of cells adapted to serial culture on glass is one started from the Ehrlich ascites tumor of mice.[1] This is a mammary carcinoma which has been induced to grow free in ascitic fluid in the peritoneal cavity of the mouse. These cells can be removed from the animal in the form of a cell suspension, thus minimizing the problems of culture. As much as 5–8 ml of fluid can be aspirated from the peritoneal cavity of a single mouse and upon centrifugation will yield up to 50% tumor cells. A variety of methods and materials has been used to start cultures of these cells, but all have been more or less modifications of the following general procedure: The fluid is removed from the mouse, treated with heparin to prevent coagulation, and spun in an angle centrifuge for about 10 min at approximately 1500 rpm. The supernatant is discarded, the cells are resuspended in 5–10 volumes of tissue culture medium, thoroughly mixed, and pipetted to Carrel D3.5 flasks in 0.5 ml amounts. These flasks are incubated for 2 hr to allow the cells to settle and adhere to the glass, after which an additional 0.5 ml of medium is added to bring the volume up to the customary 1.0 ml used on D3.5 flask cultures.

As many as 60 cultures of ascites tumor cells have been put on in half an hour, with generally 90–100% of them providing good cultures for experimental work. Some of these have been successfully subcultured by scraping, and one line at present has been maintained in serial culture for over 8 months.

Morphologically, in culture, these cells closely resemble those of the Walker rat carcinoma except that they are slightly smaller and somewhat more elongated. It seems quite possible that they are epithelial cells. All attempts to culture the cells in or on a plasma clot have failed completely, and growth on cellophane has been only slight and of short duration. These ascites tumor cells seem to prefer cultivation on glass and grow best in stationary or quiet cultures, as growth in roller tubes, even on glass, has been very poor.

The first-described technique for the establishment of animal cells in long-term culture on glass has worked successfully in this laboratory for cells from both normal and malignant mouse tissues. Further studies are in progress in which these procedures are

[1] This tumor was obtained through the courtesy of H. N. Christensen, Tufts College Medical School.

being employed in an endeavor to obtain similar strains of various cell types from human and other animal species. The publication of these results, as well as a more detailed report on the cell strains mentioned in this paper, is anticipated in the near future.

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Studies on the Antidiuretic Action of Morphine[1]

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The antidiuretic action of morphine is a well-documented phenomenon (*1*), both clinically and experimentally. The mechanism by which morphine influences renal function, however, has been a matter of some controversy. Recent evidence (*2, 3*) favors the view that morphine directly stimulates the supraoptico-posterior lobe system of the pituitary gland, causing release of the antidiuretic hormone. Despite this recurrent thesis, no efforts have yet been directed toward characterizing the urinary antidiuretic substance produced by the administration of morphine, in order to compare its properties with those of the antidiuretic hormone.

We have investigated this hypothesis of origin indirectly in normal and hypophysectomized rats receiving morphine. Experiments were conducted to demonstrate the presence of the antidiuretic substance in the urine of the test animals and to characterize it chemically. These characteristics were then compared with the properties reported for the antidiuretic hormone.

The method used for the assay of antidiuretic activity was a modification of a technique published by Dicker and Ginsburg (*4*). This consisted in administering subcutaneously 1 mg morphine sulfate/kg body weight to 6–9 adult male rats in metabolism cages, and in collecting the pooled urinary output for 24 hr. The pooled urine, either in its native state or after the various chemical and physical treatments described, was dialyzed (Visking cellulose casing, wall thickness 0.00072 in.) in running tap water for 10 hr and in distilled water for 1 hr. The dialyzed urine was then concentrated to its original volume and assayed directly. The assay animals consisted of two groups of 4 adult male rats (150–250 g), (*a*) the control group receiving 1 ml isotonic saline/100 g body weight injected subcutaneously, and (*b*) the test group receiving 1 ml of the prepared urine/100 g by the same route. These animals were hydrated with specific quantities of water (5% of their body weight), and their urine output recorded at 60, 90, and 120 min.

[1] This work was aided by the James Hudson Brown Memorial Fund of the Yale University School of Medicine.

TABLE 1

Material assayed	Mean percentage water excreted at 120 min (saline control = 100%)
Urine from unmedicated rats	99*
Pitressin (1.5 milli-u/100 g)	40†
Urine from rats receiving morphine	
Expt. 1	60†
2	59†
3	71
4	59
Urine from hypophysectomized rats receiving morphine	
Expt. 1	98*
2	94*

* Analysis of variance revealed no significant difference between this urine and the saline control.
† Analysis of variance revealed a highly significant difference between this urine and the saline control.

The data from the experiments designed to demonstrate the presence of antidiuretic activity in the urine of rats receiving morphine are summarized in Table 1. The effect recorded is the percentage of the water given to hydrate the test rats, which was excreted within 2 hr, in relation to a comparable volume excreted by the control rats, adjusted to 100%. It is interesting to note that, although morphine consistently produced urine with distinct antidiuretic potency, neither Demerol (10 mg/kg given subcutaneously at 6-hr intervals for 1 day) nor methadone (0.5 mg/kg given subcutaneously at 6-hr intervals for 1 day) had a similar action. No attempt was made in

TABLE 2

Treatment of urine from rats receiving morphine sulfate	Mean percentage water excreted at 120 min (saline control = 100%)
Untreated urine (control)	62
Heat (constant volume)*	105
Heat (to dryness)†	109
Acid hydrolysis‡	112
Alkaline hydrolysis§	118
Sodium sulfite‖	
Expt. 1	101
2	85
3	85

* Refluxed for 10 hr at 60° C.
† Incubated in an open container for 10 hr at 60° C, then restored to original volume and filtered.
‡ Refluxed in 10% HCl (5 ml acid to 50 ml urine) for 3 hr at 100° C.
§ Refluxed in 2 *N* NaOH (2.5 ml base to 30 ml urine) for 30 min at 100° C.
‖ Treated at room temperature with *N*/10 sodium sulfite (5 ml of this solution to 30 ml urine) for 5 min.

this work to study quantitatively the potency of morphine in this respect, but these preliminary data reveal that morphine produces urine with about 0.6 times the antidiuretic potency of 1.5 milli-units of pitressin/100 g body weight (tested in a similar manner). Moreover, the absence of this effect of morphine when injected into hypophysectomized rats strengthens the hypothesis that the origin of the antidiuretic substance found in the urine of normal rats receiving morphine is the neurohypophysis.

Table 2 demonstrates the effects of various physical and chemical treatments on the antidiuretic activity of urine from rats receiving morphine. It is clear that all the treatments, with the exception of the sodium sulfite, destroyed the antidiuretic substance just as they are reported to destroy the antidiuretic hormone (*5, 6*). In the case of the sodium sulfite, one experiment showed complete inactivation, and two experiments revealed only partial inactivation. Gilman and Goodman have reported a similar inconsistency in their experiments on the influence of sodium sulfite on the activity of the antidiuretic hormone (*6, 7*).

We feel that these preliminary data support the view that morphine stimulates the production of an antidiuretic substance by the neurohypophysis; and, further, that this substance has properties markedly similar to those reported for the antidiuretic hormone. A more detailed qualitative and quantitative characterization of this substance is being undertaken.

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An Outbreak of Mouse-Pox (Infectious Ectromelia) in the United States: I. Presumptive Diagnosis[1]

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During the summer of 1951 an epizootic disease made its appearance for the first time in the mouse colonies of the Department of Anatomy of Yale University School of Medicine. Infected mice usually appeared in good health until a few hours or a day before death, at which time the fur became ruffled, with a characteristic puffy appearance about the face. The most constant autopsy findings were a pale, mottled liver, hemorrhagic appearance of the proximal inch or two of the small intestine, pulmonary congestion or consolidation, and hemorrhagic appearance of the base of the claws. Death of one mouse in a pen was usually followed by death of all or most of the other mice in that pen, with similar autopsy findings, over a period of a few days or a few weeks. All attempts to prevent the spread of the disease from pen to pen were futile.

At the end of 6–8 weeks the disease had spread to practically all pens of mice. Combined mortality of all the colonies of the department often exceeded 100 mice/day. In approximately 4 months after onset, the death rate had returned to a normal level. Although exact figures are not available, it is estimated that at the end of this period the total mouse population had been reduced to approximately 50% of its initial size. Of the animals alive at the end of this time, many had been born during the course of the epizootic. Of the animals existing at the onset of the disease, therefore, less than 50% survived. The mortality rate appeared to be higher among some strains (A and BC) than among others (C_{57}). One group of over 100 mice of the A strain, set aside for a particular experiment, suffered 100% mortality.

While the death rate was still increasing, foot pad inoculations were undertaken because of the similarity of some of the findings to those described by Dingle (*1*) for the acute phase of infectious ectromelia. At the time no cutaneous lesions were apparent. Liver suspensions from 5 mice believed to have the disease were injected into the foot pads of healthy mice. Four of the 5 suspensions proved infectious. The injected foot of all 18 mice receiving these 4 suspensions became inflamed and edematous, usually on the third day. Fourteen mice died, and 3 were killed when death seemed imminent, in from 4 to 7 days (av, 5.2). Smears of the injected foot failed to reveal bacteria. Autopsy findings were similar to those described above for the spontaneous disease. The eighteenth mouse survived; its injected foot became edematous, exudative, scabbed, and underwent progressive loss of the distal half, suggestive of the chronic cutaneous phase of infectious ectromelia. Several weeks later, after the death rate in the colony had returned to a low level, cutaneous lesions of the tail, feet, face, and body appeared spontaneously in many of the surviving mice.

The chorioallantoic membrane of chick embryos was inoculated with a suspension of liver from one of the 18 experimentally infected mice. Autopsy findings on this mouse were typical of the spontaneous disease. Eight of 12 inoculated eggs showed pocklike lesions of the membrane, similar to those described by Burnet (*2, 3*) for the virus of infectious ectromelia. Seven of these membranes were pooled, ground, and centrifuged. The supernatant suspension was injected into the foot pads of 7 mice. Six died on the sixth day and one on the seventh day, with swollen foot pad and autopsy findings similar to those of the spon-

[1] This investigation has been aided by grants from the Jane Coffin Childs Memorial Fund for Medical Research and the U. S. Public Health Service.

taneous disease. Of 10 mice inoculated with material from the second egg generation (after intervening lyophilization), 4 died on the fifth day and 6 on the sixth day, with swollen foot pad and autopsy findings similar to those of the spontaneous disease. This culture has been carried through 6 egg generations, with intervening storage in glycerol. Penicillin and streptomycin have been added since the third egg generation. It has continued to reproduce the same proliferative pocklike lesion of the chick membrane, and the same disease in mice not previously exposed.

Intracytoplasmic, eosinophilic inclusion bodies have been observed in tissues from both infected mice and eggs. They were, however, not as frequently found in the tissues of infected mice as might have been expected from the literature on infectious ectromelia. Moreover, because of their lack of specificity, definitive diagnosis should depend not on the presence or absence of inclusion bodies, but on serological or immunological methods.

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An Outbreak of Mouse-Pox (Infectious Ectromelia) in the United States: II. Definitive Diagnosis[1]

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The immunological similarity of the viruses of infectious ectromelia and vaccinia was first reported by Burnet (*1–3*) on the basis of the following observations:

a) The virus of infectious ectromelia, growing on the chick chorioallantois, produces a soluble hemagglutinin which agglutinates the same spectrum of fowl erythrocytes agglutinated by vaccinia hemagglutinin.

b) Both ectromelia and vaccinia hemagglutinins are inhibited by either ectromelia or vaccinia immune sera, but not by normal sera.

c) Vaccinia can be used to immunize mice effectively against ectromelia.

On this basis Burnet suggested that infectious ectromelia is the murine representative of the mammalian pox diseases, and proposed (*4*) that the term "mouse-pox" should be used as a synonym for infectious ectromelia. This work has been confirmed and considerably extended by Fenner (*5–11*), who adopted "mouse-pox" to describe the disease, but retained "ectromelia" to describe the causative virus.

Definitive diagnosis of the disease described in the preceding article (*12*) was made by demonstration of vaccinia antihemagglutinin in the sera of convalescent mice, and by successful cross-immunization with vaccinia against the isolated etiological agent.

Approximately 2 months after the mortality rate had returned to normal, 18 mice that had lived through the epizootic were bled and the sera tested for inhibition of the hemagglutinin produced by the Nelson and Levaditi strains of vaccinia virus. Six of the sera inhibited 4 agglutinating doses of vaccinia hemagglutinin at dilutions of 1–20 to 1–80. Nine of the sera showed no inhibition of vaccinia hemagglutinin at a dilution of 1–10. At the same time 14 sera from a group of mice separated from the main colony during the course of the epizootic, but before showing manifestations of the disease, were tested. One showed inhibition of vaccinia hemagglutinin at a dilution of 1–48. The remaining 13 were negative at a dilution of 1–6.

TABLE 1

Cross-Immunization by Vaccinia (Nelson) against the Isolated Etiological Agent

Intranasal vaccinia	No. of mice	Specific mortality following foot pad inoculation 19 days later
None	30	20/30
1–100 Dilution	29	6/29
1–5 Dilution	29	3/29

For cross-immunization, mice were used from a source without previous known exposure to either vaccinia or ectromelia. Two groups received intranasal inoculation, under ether anesthesia, of vaccinia (Nelson) at dilutions of 1–5 and 1–100. A third group received no vaccinia. Nineteen days later all three groups were challenged by foot pad inoculation of the etiological agent, isolated as previously described (*12*). Those groups exposed to vaccinia showed definite protection (Table 1). The experiment was repeated, using a different strain of vaccinia (Levaditi) and a 40-day interval between intranasal inoculation and foot pad injection. The mice receiving vaccinia were again protected (Table 2).

Mouse-pox is enzootic in laboratory mice in Europe. The U. S. has been remarkably free of the disease. Importation of the virus is prohibited by the U. S. Bureau of Animal Industry (*11*). Serological surveys for the presence of vaccinia antihemagglutinin in mice from different breeding stocks in the neighborhood of New York City and Boston failed to reveal a single positive result (*11*).

Fenner (*11*) quotes two references (*13, 14*) and a personal communication (*15*) suggesting that the disease may previously have existed in the U. S. The

[1] This investigation has been aided by grants from the Jane Coffin Childs Memorial Fund for Medical Research and the U. S. Public Health Service.

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first is extremely vague. The second is strongly suggestive. The third refers to endemic "ectromelia" in one of the mouse colonies of the Department of Anatomy of this institution. This suggests the possibility that the present outbreak may represent a sudden flare-up of a pre-existing disease. Fortunately, evidence is available regarding this plausible possibility. The term "ectromelia" has been used loosely in this department to refer to gangrenous lesions of the feet or tail, which sometimes resulted in loss of the extremities. Such lesions have appeared in small numbers at widely separated times over a period of many years, and have been associated with a low mortality rate. Such lesions may be caused by agents other than ectromelia virus (*16, 17*). No conclusive evidence exists that the lesions seen in this department prior to 1951 were caused by the virus immunologically related to vaccinia. On the contrary, evidence exists that they were not. Approximately 60 sera from a number of inbred strains of mice in the department were tested for vaccinia antihemagglutinin in 1948 and 1949, with negative results. On a number of occasions since 1949, but prior to the outbreak in 1951, mice bearing gangrenous lesions of the feet or tail have been examined.

TABLE 2

CROSS-IMMUNIZATION BY VACCINIA (LEVADITI) AGAINST THE ISOLATED ETIOLOGICAL AGENT

Intranasal vaccinia	No. of mice	Specific mortality following foot pad inoculation 40 days later
None	15	11/15
1–5 Dilution	14	0/14

The sera from these mice did not contain vaccinia antihemagglutinin. Serial passage in mice of an extract from these lesions failed to induce any signs of an infection when injected intraperitoneally or into the foot pad, and no pocks were produced when the extract was inoculated on the chorioallantois of the chick embryo.

Because of confusion resulting from the use of the term "ectromelia," or even "infectious ectromelia," it is strongly recommended that the suggestion of Burnet and Fenner be universally adopted, and the term "mouse-pox" be used to refer to the disease of mice caused by the virus related to vaccinia.

If the virus of mouse-pox was not present in the Department of Anatomy prior to 1951, whence then did it originate? After the disease had made its apperance and had been diagnosed in this department, it was learned that an outbreak of what was apparently the same disease had occurred in another department of the university a year earlier, with loss of approximately 60% of that colony. It is known that mice were transferred from that colony to the Department of Anatomy approximately 2 months before the 1951 outbreak was noted.

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A Simplified Preparation of High-Polymer Desoxyribonucleic Acid

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High-polymer desoxyribonucleic acid has customarily been prepared by the removal of contaminating protein from nucleoproteins extracted in strong salt solutions. Until recently the deproteinization has been accomplished by shaking with chloroform and centrifuging, according to the method described by Sevag *et al.* (*1*). More recently deproteinization procedures based on the removal of proteins by precipitation with detergents have been described (*2, 3*). The chief drawback in either case is that these are laborious and time-consuming, requiring several days for their completion. By combining the chloroform and detergent methods, a simple, rapid, and highly satisfactory procedure has been developed.

The source material (isolated nuclei or sperm) is extracted in 2.0 *M* NaCl, in the cold, and all subsequent operations are done in the cold. When the thick, viscous nucleic acid and protein extract is obtained, an equal volume of reagent grade chloroform is added, the material is shaken violently by hand or mixed in a Waring blendor at a slow speed. However formed, the resulting emulsion is centrifuged and the aqueous supernatant layer is decanted from the protein pad and the chloroform. To the aqueous supernatant solution, 1.25 volumes 1% detergent (Duponol C; Na dodecyl sulfate) are added, and then additional 2.0 *M* NaCl to give a final concentration of 1.0 *M* NaCl, and 0.5% detergent. The preparation now contains a white, murky precipitate that can be removed by centrifugation. Ultracentrifugation ($25{,}000 \times g$ for 30

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min) at this step, although not essential, is helpful in removing very finely precipitated particles. The clear supernatant liquid is precipitated in 4 volumes 95% ethyl alcohol, the fibrous precipitate is washed in more alcohol, then in absolute alcohol, and finally in ether, and dried.

Further treatments are in large measure determined by the properties of the different preparations and of the individual making the preparation. In general, the material should be redissolved in 2.0 *M* NaCl, and treated again with the detergent, centrifuged, and precipitated in alcohol, then dissolved in distilled water, and precipitated with alcohol. This final alcohol precipitation should be repeated at least three times.

The product obtained is white, fibrous, hygroscopic, and readily soluble in water, to give a perfectly clear but viscous solution.

The principal merits of the preparation lie in the exploitation of the capacity of the chloroform and of the efficiency of the detergent for the removal of protein from the DNA–protein extract. The efficiency of the chloroform treatment will determine the necessary extent of the detergent treatment; the alcohol precipitations are a final purification, primarily for the removal of the detergent.

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The Existence of Antifibrinolysin Activity in Platelets[1]

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Recent studies of fibrinolysin (plasmin) and antifibrinolysin (antiplasmin) have advanced our knowledge of this enzyme system (*1–6*) considerably, owing largely to chemical purification work and methods for quantitative measurement of activity. Plasma of most animals examined has been found to contain profibrinolysin and antifibrinolysin (*7*). Quantitative studies show that antifibrinolysin concentration of plasma may fluctuate in various diseased states, and especially interesting is the rise in its concentration with pteroylglutamic acid deficiency in chicks (*1, 2*).

Apparently, in all the interesting studies of plasma, no one has attempted to find, or by chance found, antifibrinolysin in platelets or other formed elements. Its existence in platelets came to our attention when we were studying the platelet clottable factor which is evident in our platelet extracts. It was of interest to determine whether the clottable factor of platelets is fibrinogen itself or a substance with properties nearly like fibrinogen. Among other things, in pursuit of this problem, an attempt was made to lyse the platelet clottable material with purified fibrinolysin, prepared by Loomis (*8*). It was found that the clottable factor would not lyse even though strong solutions of purified fibrinolysin were mixed with it. This finding could be interpreted to mean that the clottable factor was itself resistant to fibrinolysin. Another possible interpretation, however, was that the platelet clottable factor preparation contained antifibrinolysin, and subsequently many experiments have confirmed this.

In a number of experiments an attempt was then made to determine the quantity of antifibrinolysin present in platelets and to compare this quantity with the concentration of normal plasma. For this purpose, bovine materials were used throughout. Platelets were obtained by commonly used differential centrifugation procedures, and a suspension in physiological saline solution was made. This suspension contained about 30 times more platelets, by actual count with the phase contrast microscope, than are found in cow blood. This platelet suspension was frozen and subsequently thawed. This freezing and thawing, in our experience, is an effective way of liberating materials of platelets. The thawed preparation was lightly centrifuged to remove any particulate material. A few units of purified thrombin (*9*) were then added to clot out the so-called clottable factor first found to be in platelet extracts by Ware, Fahey, and Seegers (*10*), all added reagents having been found to contain no apparent antifibrinolysin activity. The assay (*1*) for antifibrinolysin content of this platelet preparation showed that it contained 2310 u antifibrinolysin/ml. Quantitative data on the various volumes involved were carefully kept. Simultaneously, oxalated bovine plasma was found to contain 222 u antifibrinolysin/ml. This is approximately the same as found by Guest, Ware, and Seegers (*1*). By assuming a hematocrit of 45% and disregarding a correction for anticoagulant volume, it was possible to estimate the distribution of antifibrinolysin activity in plasma and platelets. The platelet compartment contained approximately 70% as much antifibrinolysin as was obtained from the plasma of the same sample of blood. Even though the experiment was done several times, this cannot be regarded as an exact quantitative statement, but, rather, as an order of magnitude concept, because there were some quantitative considerations that could not be taken into exact account.

From our experiments there is no way of knowing whether the antifibrinolysin of platelets is the same substance as that of the plasma. However, the discovery of antifibrinolysin activity in platelets brings up that question for future consideration, for it is

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possible that the antifibrinolysin activity of plasma is derived from the platelets.

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Protection Against Alloxan Diabetes by Mannose and Fructose

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In a previous communication (*1*) from this laboratory, it was reported that glucose, given prior to the injection of diabetogenic doses of alloxan, prevented diabetes in rats.[2] The present communication reports the results of similar experiments done with the other physiologically important hexoses—namely, mannose, fructose, and galactose.

Rats fasted overnight were given diabetogenic doses (40 mg/kg) of alloxan intravenously at different intervals of time after intravenous administration of varying doses of the above sugars. Blood sugar values of the rats were then determined daily for 7 days. The urine was also tested daily for the presence of sugar during the experimental period. A blood sugar value over 180 mg% for 2 consecutive days and the presence of sugar in the urine for a similar period of time were considered to indicate a diabetic condition in the animals. The results are given in Table 1.

Thus mannose and fructose, given prior to the injection of diabetogenic doses of alloxan, prevented diabetes in rats. Galactose had no such preventive action. The present results, considered together with those given in the previous communication (*1*), show that the preventive abilities of glucose and mannose are practically of the same order. Mannose is possibly slightly less effective than glucose. Fructose, however, is obviously much less effective.

Inhibition of essential sulfhydryl enzymes of the β-cells has been suggested as the cause of the diabetogenic action of alloxan (*2*). Reversal of the action of alloxan by sulfhydryl compounds (*3*) has added strength to this hypothesis. Mammalian hexokinase is a sulfhydryl enzyme, being inactivated by alloxan and reactivated by cysteine (*4*). There is considerable evidence (*5, 6*) that this enzyme is fundamentally related to the condition of diabetes in animals—the activity of the enzyme being inhibited in diabetes. Hexokinase is a nonspecific enzyme acting on glucose, mannose, or fructose. The affinity of fructose for the enzyme is, however, low compared to that of glucose or mannose for it (*7*). In addition to the above nonspecific hexokinase, mammalian tissues may contain a specific fructokinase, which does not act on glucose or mannose (*7*). Galactose is attacked by another specific enzyme, galactokinase (*8*). The ordinary hexokinase is presumably present in the β-cells; for, like all other cells, the β-cells must also ordinarily obtain energy from the oxidation of glucose, the first step in the process being catalyzed by the above enzyme.

TABLE 1

EFFECT OF PREVIOUS ADMINISTRATION OF MANNOSE, FRUCTOSE, AND GALACTOSE ON THE PREVENTION OF DIABETES CAUSED BY INTRAVENOUS ALLOXAN (40 MG/KG) IN RATS

Sugar injected	Dose of sugar injected (g/kg)	Interval between injection of sugar and alloxan (min)	No. rats used	No. diabetic rats
Mannose	1	5	6	0
	1	10	6	5
	2	5	5	0
	2	10	6	0
	2	15	6	6
Fructose	2	5	5	5
	5	5	6	0
Galactose	2	5	5	5
	5	5	5	5

The results obtained show that the relative abilities of the hexoses to prevent the diabetogenic action of alloxan are directly comparable to their affinities for hexokinase.[3] They also show a close similarity with the results obtained on the protection of yeast hexokinase against inactivation by proteolytic enzymes by the above sugars (*7*). One therefore wonders whether the protection against the diabetogenic action of alloxan by glucose, mannose, and fructose is due to a protection of the hexokinase in the β-cells against alloxan poisoning and whether inhibition of β-cell hexokinase is the primary cause of the diabetogenic action of alloxan. It is also interesting to speculate whether the results reported here offer any clue to the understanding of the selectivity of alloxan for the

[1] I am greatly indebted to P. B. Sen for advice and criticism and for the grant of laboratory facilities. My thanks are due to the Lady Tata Memorial Trust, Bombay, for the grant of a research scholarship.

[2] Similar results (unpublished) have been obtained with rabbits.

[3] It may also be noted that the relative abilities of the hexoses to protect against alloxan diabetes are directly comparable to their effectiveness to alleviate hypoglycemia in animals.

β-cells. The β-cells synthesize and store insulin. It is therefore likely that the concentration of glucose in these cells will be lower than elsewhere in the body. A specifically low concentration of glucose in the β-cells may, in view of the above results, account for the selectivity of alloxan for these cells.

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The Attempted Dehydrogenation of 3,4-Disubstituted Thiolanes[1]

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We have subjected 3,4-thiolanediol and its diester and diethyl ether derivatives to dehydrogenation procedures in an attempt to prepare the corresponding thiophene analogs. Thiolane itself has been dehydrogenated to thiophene in 32% yield, using platinized charcoal at 400°, and in 18% yield with nickel sulfide on alumina at 350° (*1*); in each case the remainder of the material was converted to hydrogen, hydrogen sulfide, olefins, and alkanes. Passage of thiolane vapors through a "red-hot" glass tube is reported to give traces of thiophene (*2*). The syntheses of thiophene and its homologs from hydrocarbons and sulfur probably involve thiolane intermediates which subsequently undergo dehydrogenation (*3*), and sulfur and thiolane under pressure do give small yields of thiophene (*4*).

DL-1,4-Dichloro-2,3-dihydroxybutane (*5*) was cyclized to 3,4-dihydroxythiolane (*6*) with sodium sulfide. The latter was converted to the diacetate and dibenzoate esters. The diacetate was also prepared by cyclizing DL-1,4-dichloro-2,3-diacetoxybutane (*5*). 3,4-Diethoxythiolane was prepared by a slight modification of the method of Patterson and Karabinos (*7*).

The four substituted thiolanes were heated with sulfur under dehydrogenating conditions with and without solvents. When short reaction times were employed, most of the starting material was recovered unchanged. Under more vigorous conditions decomposition occurred, with the concomitant evolution of hydrogen sulfide. Similar results were obtained using platinum on charcoal as the dehydrogenating agent. Neither of the diesters was attacked by chloranil.

In a series of vapor phase runs, solutions of sulfur and the dibenzoate or the ether were passed under nitrogen pressure through a tube packed with "non-catalytic" fused alumina balls[4] and maintained at temperatures varying from 450° to 525°. Decomposition occurred in every case. Attempts to split out two molecules of acid from the diester by pyrolysis in the absence of sulfur (*8*) were also unsuccessful.

We believe that dehydrogenation took place during these various experiments, and that the substituted thiophenes then decomposed. The instability of 3,4-dihydroxythiophene has been mentioned by Fager (*9*) and by Turnbull (*10*). The ethoxy, benzoxy, and acetoxy substituents would tend to increase the susceptibility of the thiophene nucleus to degradative attack because of their action in increasing the electron density in the ring.

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[4] We wish to thank the Aluminum Company of America for the gift of this material.

The Response of Two Species of Pine to Various Levels of Nutrient Zinc[1]

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The effect of a lack of nutrient zinc on the growth of a number of forest tree species has been reported by several workers (*1–4*). In general, the symptoms of all the species studied have been quite similar, being subsumed under the heading of little-leaf or rosette disease. These names are quite descriptive, since the affected trees usually exhibit diminished bud growth, reduction of leaf size, and chlorosis of varying degrees of severity. The symptoms are also concordant with the known effect of a lack of zinc in reducing the auxin level of the deficient plants. Although naturally occurring zinc deficiencies have been

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[1] This work has been assisted by a grant from the Tennessee Corp.

demonstrated to exist in citrus and tung trees in California and Florida and in pecan trees in Georgia, the only known reference to a natural deficiency in pine trees was reported for *Pinus radiata* in Australia (*1*). Subsequent studies performed on seedling plants of this species growing in culture solution established the need of zinc for healthy growth (*5*). The deficiency symptoms of the seedling plants were similar to those observed on trees growing in zinc-deficient soil and included diminished bud growth, shortened needles, and varying degrees of chlorosis.

It has been reported that a serious deterioration of shortleaf pine (*Pinus echinata* Mill.) and to a lesser extent loblolly pine (*Pinus taeda* L.) was prevalent throughout the Piedmont region (*6*). This deterioration, which became known as the little-leaf disease, occurs extensively on shortleaf pine, and to a lesser extent on loblolly pine when associated with shortleaf pine. The little-leaf disease is regarded as a systemic disturbance that causes a gradual reduction of the growth of all the aboveground parts of the affected tree. Needle length decreases from a normal of 3–4 in. to a minimum of 1 in., or even less in very acute instances. Growth of new shoots declines from a length of 7–12 in. to a minimum of 1 in. or less. Reduction of growth is accompanied by a gradual increase in the degree of yellowing, reaching a definite chlorosis in the acute stages of the disease.

Although the symptoms of the trees affected with the little-leaf disease are similar to those reported for the zinc-deficient trees of *P. radiata*, the writer is unaware of any published reports of the effect of zinc on the growth of either loblolly or shortleaf pine. Such a study was undertaken and will be reported briefly.

A sufficient quantity of seeds of both loblolly and shortleaf pine were stratified in dishes of moist sand and placed in a refrigerator maintained at 3° C for a period of 2 months. They were then planted in acid-washed quartz sand and after 2 months were transplanted to especially prepared containers.

The plants were grown in 2-liter Pyrex beakers that had previously been painted on the outside with a coat of black asphaltum, followed by a layer of aluminum paint. To permit drainage a small hole was bored in the bottom of each beaker, and a piece of Pyrex wool placed over the hole. Fine quartz sand was washed with concentrated sulfuric acid and rinsed with Pyrex-distilled water. The sand was then placed in the beakers and washed with a 1 : 1 solution of hydrochloric acid, followed with a thorough rinsing with Pyrex-distilled water. Cardboard tops, impregnated with paraffin and punctured with several small holes, were used as covers for the beakers.

Nutrient solutions were prepared and purified separately, 5 liters at a time, in 6-liter flasks. "Major" nutrients were prepared, using the methods of Arnon (*7*) and Stout and Arnon (*8*), in which molar solutions of the salts were used. The nutrient solutions and distilled water used in the experiments were tested frequently by the dithizone test, as described by Stout and Arnon (*8*), and found to contain less than 1 part in 10 billion of trace metals. A trace supplement, which consisted of boron, .5 ppm; copper, .02 ppm; manganese, .5 ppm; and a .5 % iron solution, was used. Concentrations of zinc of .000 ppm, .001 ppm, .01 ppm, and .1 ppm were used.

One plant of loblolly and one plant of shortleaf pine were planted in each of four beakers in each of the four concentrations. It was considered that by having the two species growing in the same containers there would be less likelihood of fortuitous results. The containers were saturated with nutrient solutions twice a week, and the plants watered with distilled water in the intervening time. The plants were allowed to grow in an unshaded greenhouse for approximately a year.

At the end of the year there was a great difference in the response of the two species. All the loblolly seedlings were still alive and growing, although the growth response showed a definite effect of the various concentrations of zinc. The plants in the minus zinc series were about half as tall as the plants in the high zinc series, had shorter needles, and exhibited some chlorosis. The plants growing in the two intermediate concentrations of zinc exhibited some diminution of terminal bud growth, some reduction of needle extension, and a slight chlorosis when compared with the controls.

The shortleaf seedlings growing in the minus zinc series were all dead at the end of 8 months. They had made practically no growth, the needles were very short, and the plants exhibited extreme chlorosis. The .001 ppm series contained three dead and one weakly living plant at the end of one year. The plants had lived a few months longer than those in the minus zinc series and had made slightly more terminal bud growth, but they also showed failure of needle extension and severe chlorosis. In the .01 ppm series only one of the plants was dead, but the remaining seedlings exhibited diminished bud growth, greatly reduced needle extension, and severe chlorosis. In the high zinc series the plants were vigorously alive and exhibited normal healthy growth.

It will be noted in Table 1 that, although the height growth of loblolly shows a definite relation to

TABLE 1

EFFECT OF VARIOUS CONCENTRATIONS OF NUTRIENT ZINC UPON THE GROWTH IN LENGTH (IN CM) OF LOBLOLLY AND SHORTLEAF PINE SEEDLINGS

	Concentrations of zinc in ppm							
	.000		.001		.010		.100	
	Shortleaf	Loblolly	Shortleaf	Loblolly	Shortleaf	Loblolly	Shortleaf	Loblolly
	10	23	11	23	10	28	28	28
	5	22	10	24	17	28	28	44
	3	20	8	23	14–	32	30	35
	5	18	11	27	14	30	26	36
Av	6	21	10	24	14	30	28	36

the effect of various concentrations of zinc, there was fairly good growth in the low zinc series. However, there was practically no growth of shortleaf pine in any concentration below the control.

These data clearly indicate that in addition to *P. radiata*, both loblolly and shortleaf pine require zinc at approximately .1 ppm for continued normal growth. In addition, there appears to be a marked difference in the nutrient requirements of loblolly and shortleaf. The symptoms of zinc deficiency produced in both species closely resemble those described for the little-leaf disease that has been reported to affect shortleaf and to a lesser extent loblolly in the Piedmont area (*6*). As to whether the little-leaf disease is in truth a zinc deficiency this experiment is not designed to answer. It may be that the disease is caused by factors such as fungi, nematodes, poor aeration, etc., which affect the absorption of zinc, with consequent production of zinc-deficiency symptoms.

Perhaps the most striking result of the experiment is the establishment of such great differences in the response of the two species to the various levels of zinc. The difference is of such magnitude as to suggest a basic difference in various physiologic processes. More extended investigations are currently in progress; they will deal not only with the effect of other nutrient elements but also with the effect of the presence or absence of mycorrhiza.

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Comments and Communications

Scientific Documentation through Cooperation

Some comment should be made on the note by Jacques Avias on "International Organization of Scientific Documentation Based on Legislation" (Science, **115**, 250 [1952].) It happens that my fields of special study (paleontology and stratigraphic geology) are the same as those of M. Avias, and, like him, I am concerned with the problem of effective dissemination of knowledge in our subjects.

M. Avias would have laws forbidding scientific work to circulate unless accompanied by cards of standard size with abstracts in specified languages, unless special cards are deposited with specified international agencies, and so on. I find the thought of such coercive legislation very repugnant, and more than a little frightening. It would be unfortunate were our colleagues in other scientific fields left with the impression that bibliographic conditions in paleontology are so chaotic that such bureaucratic authoritarianism is warranted. The actual conditions are not ideal, but they are not thoroughly bad.

The geological (including paleontological) literature pertaining to North America, in its broadest sense, is listed in annual bibliographies with analytical indices, published by the U. S. Geological Survey and available to all at a very small cost. These bibliographies are amazingly complete, and are published as promptly as could be expected: the volume for a given calendar year appears during the next year, and commonly during the next summer.

For the literature of the rest of the world one must look farther, and coverage is not so perfect, but I think that comparatively few papers escape notice in the *Bibliography and Index of Geology Exclusive of North America*, published annually by the Geological Society of America, Schindewolf's *Zentralblatt für Geologie und Paläontologie* (happily again appearing regularly, with admirable reviews), or the *Bulletin analytique*, the geological parts of which are reprinted in an annual volume by the Société géologique de France. Besides these inclusive listings, almost all countries have some agency publishing summaries of work of more local interest, such as the annual review of Swedish papers, which appears as part of the *Förhandlingar* of the Geologiska Föreningen i Stockholm. By consulting half a dozen sources (surely not an impossible task for a research worker), well over 90% of all papers in geology and paleontology will be found. For papers dealing with paleontology, abstracts and analyses are found in *Biological Abstracts* and the *Zoological Record*, as well as in the *Zentralblätter*. Coverage here may be described as good, although improvable and improving.

This result has been accomplished through no armament of laws, but by the geologists and paleontologists of the world working together through their professional societies, or through looser organizations like *Biological Abstracts*. No statute demands that copies of books or journals be sent to editors, although no doubt the editors are pleased, and their work is lightened, when this is done. I do not believe we need be ashamed of what has been accomplished bibliographically by free cooperation and informal zeal.

Granted that a worker may thus learn the titles of almost all papers in his field, does he then need the batteries of especially trained translators and the implied miles of filing cabinets in Paris (presumably in

the care of especially trained librarians), which M. Avias suggests? I think not.

If I find that a potentially interesting article has appeared, say, in the *Doklady* of the Russian Academy of Sciences, which we do not have in Delaware, am I blocked in my research unless some law compels the academy to provide me with a 3″ × 5″ card bearing an abstract in Spanish? Not at all. I go across the street to our librarian, ask him to borrow this issue for me on interlibrary loan, and he does so, with no fuss and little delay, because the librarians of this continent have pooled information on their holdings and accessions, and have agreed to lend to one another books needed for scholarly work. There are no laws and no top-heavy organization, but practically any book in existence is available to any worker, no matter how small or inadequate his local library may be. I can imagine no bureaucratic center for documentation serving scientists more efficiently, more smoothly, and more cheaply than our librarians now do.

For my European colleagues may I add, this is possible not only in a country with many libraries and no hampering political boundaries. While I was working in Canada, I could borrow books as readily from Harvard as I now can in Ohio.

Let us look around and see what has been accomplished by cooperation, and let us work to make this cooperation more effective. Without arrogance but with pride, let us show the results to those who claim that only through compulsion can scientific work flourish.

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Has He a Rival?

Who was the youngest person ever elected to membership in the AAAS? Probably it was William Fellowes Morgan, a grandnephew of Lewis Henry Morgan, the father of American anthropology.

The January 1945 *AAAS Bulletin* contains an article entitled "The Six Patriarchs of the Association," listing six members (at that time all deceased but one) each of whom had been members of the Association for at least 64 years.

One of the six is William Fellowes Morgan (1860–1943), who was a member of the AAAS for 65 years. He was elected a member in August 1878, when he was only 17 years old (his 18th birthday was on the following September 24). He was probably the youngest member the AAAS has ever had. He was elected a fellow of the AAAS in 1932 and an emeritus life member in 1933. He was associated with Section M. In 1880, William Fellowes Morgan was a member of the Association while his granduncle was president.

In addition to blood relationship and membership in the AAAS, William Fellowes Morgan and Lewis Henry Morgan were both successful businessmen and both trustees of Wells College. In 1868, the year that Wells College (founded by Henry Wells, who was also a founder of Wells, Fargo & Co. just a century ago) opened, Lewis Henry Morgan was elected a trustee and served until his death in 1881. William Fellowes Morgan was a trustee for 25 years, for 13 of which (1927–39) he was chairman of the board.

Temple R. Hollcroft

Aurora, New York

Express Rates or Animals?

The Railway Express Co. has applied to the Interstate Commerce Commission for another rate increase, of *25%*. Since shipments by Railway Express of live materials, especially of mammals, are already penalized with twice the first-class rate, to which several increases during the past few years have been added, it will be a very costly proposition for researchers to use Railway Express.

So that the increase shall not be irrevocably passed by the Interstate Commerce Commission, I would like to call the bad situation to your attention, with the suggestion that you might alert colleges, universities, hospitals, and medical schools so that protests are made *in time*.

The rates are absolutely unreasonable. I get many complaints about the high costs of live research materials, but the doctors do not realize that in many shipments the railway expressing costs are higher than our materials. The Railway Express Co. should be brought to reduce the rate to that of other common materials or assume responsibility for proper handling—then the rate increase might be justified.

Unfortunately, we cannot ship large mammals by Air Express because the embargoes have been revoked only for smaller shipments. What is needed is stiff competition, so that they would have to give better service instead of asking for one increase in rates after another.

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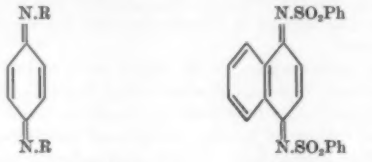
Reactions with *p*-Quinone Imides

Recently, Adams and co-workers (*1*) have studied the additive reactions of *p*-quinone imides—e.g., with thiophenol, amines, various organic acids, and aromatic hydrocarbons.

As far as we are aware, the action of the Grignard reagents on *p*-quinone imides has not been investigated. We have found that 1 : 4-naphthoquinone dibenzenesulphonimide (II) reacts in an analogous way with ethyl- and phenylmagnesium halides to form the colorless additive products (mp 186°) from benzene (C, 61.7%; H, 4.7%; N, 5.9%; S, 13.5%; $C_{24}H_{22}N_2O_4S_2$ requires C, 61.8%; H, 4.7%; N, 6.0%; S, 13.7%), (mp 220°) from benzene (C, 68.5%; H, 4.5%; N, 4.6%; S, 10.7%; $C_{28}H_{22}N_2O_4S_2$, C_6H_6 requires C, 68.9%; H, 4.7%; N, 4.7%; S, 10.8%), respectively. *p*-Quinone dibenzenesulphonimide (Ia),

2-chloro-1 : 4-naphthoquinone dibenzenesulphonimide and *p*-quinone dibenzimide (I*b*) undergo reduction to their corresponding amides when allowed to react with methyl- and ethylmagnesium halides. The reduction of the C=N by the Grignard solutions is a new example of the reducing action of Grignard reagent. (I*a*,) (I*b*), and (II) are readily reduced by magnesium-magnesium iodide mixture to the corresponding *p*-quinone amides.



I*a*, R = SO_2Ph; I*b*, R = COPh II

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Removal of Soft Parts of Snails by Freezing

THE troublesome problem of removing soft parts of fresh-water gastropods from their shells may be solved by using the following simple technique, which has not been previously reported. The living specimen, covered with water in a stoppered lip or shell vial, is placed in a freezing unit. After the water has frozen completely, the vial is removed to permit thawing at room temperature. The soft parts can then be extracted easily with forceps or dissecting needles.

The method was used successfully on many scores of specimens representing twelve species of fresh-water gastropods. Its advantages over previously reported methods (*1*) are: (1) soft parts may be removed *in toto*, (2) the viscera remain intact for possible morphological study, and (3) radulae and jaws are easily expelled by gentle downward pressure with a dissecting needle on the dorsal, posterior portion of the cephalic region.

The method is an improvement over those used by former workers in the field.

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Reference

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A Rapid Technique for the Diagnosis of Intestinal Protozoa[1]

FOR the past several months a new and simple technique for the rapid diagnosis of intestinal protozoa in feces with a single solution (tincture Metaphen, Abbott—tinted alcohol-acetone aqueous solution, 1:200) has been on trial in the parasitology laboratory of this activity. Because of the excellent results obtained, it is believed to be superior to other wet-staining techniques currently in use. With this method both the cysts and trophozoites of the intestinal protozoa can be clearly demonstrated.

The technique is as follows: A drop of stain, not diluted with saline or other solution, is placed on a glass microslide. A small particle of feces is stirred in until a smooth emulsion is formed. It is now ready to be examined under the low-power objective, the protozoa being readily detected, as they appear highly refractile. It is then necessary to change to either high dry or oil immersion for finer detail of the organism; high dry is usually sufficient. Better results are obtained when the feces are fresh, yet even formalin-preserved specimens are readily stained.

With this stain the nuclear and cytoplasmic details of the cysts are readily diagnostic. The cytoplasm assumes a greenish-yellow coloration, and the nuclei are in distinct contrast, with the jet-black granules of the nuclear ring clearly defined. The karyosome is likewise well demonstrated, this being of value in the differentiation of *Endamoeba coli* and *E. histolytica*. Chromatoid bars are clear and distinct.

The staining characteristics of the trophozoites are similar to that of the cysts. The pseudopodia are immediately fixed upon contact with the stain. Ingested erythrocytes and bacteria are in sharp contrast to the surrounding cytoplasm.

Unfortunately, *Dientamoeba fragilis* has not been observed, but the above observations are consistently true for the other ameba of man. The nuclear morphology and flagella of the intestinal flagellates are distinct, this being especially true with *Giardia lamblia*.

The advantages of this stain are multiple. The technique should be of value to all small laboratories, where facilities are limited. Its extreme simplicity allows for a rapid diagnosis of all the intestinal protozoa of man. It has also been found to be diagnostic for the rhabditiform larvae of hookworm and *Strongyloides*. The solution is stable and will not deteriorate on long standing. It is readily available and relatively inexpensive in comparison with other staining solutions.

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[1] The opinions contained in this paper are those of the authors. They are not to be construed as necessarily reflecting the views or the endorsement of the Navy Department.

Hydraulic Ram Action of Kidney Glomerulus

IT HAS been brought to my attention by Professor Swann, of Galveston, that a suggestion communicated by me (SCIENCE, **114**, 505 [1951]) regarding the possible hydraulic ram action of the kidney glomerulus is antedated by an elegant article in the *New Orleans Medical and Surgical Journal* of May 1880, by J. G., attributing the concept to Andrew Smyth. Smyth based the ram action on occlusion of the efferent arteriole by the afferent pulsation and used it to deny Bowman's claims regarding filtration.

A later suggestion of intrinsic contractile power of the glomerular tuft (*J. physiol.* [Paris], **7**, 660 [1906]) does not seem to fit the histology, although any one of them would serve to produce the missing component of the secretory pressure of the nephron. Only more adequate analysis of the orders of magnitude of the theoretical effects can validate their importance.

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Cosmic Cloud Hypotheses of the Origin of the Solar System

> The public is being played upon and utterly misled by the dreamery of the rival mathematical astronomers and physicists.

THIS statement by Henry E. Armstrong (*1*) quoted by Van Anda (*2*) in SCIENCE 21 years ago in his "Unsolved Riddle of the Solar System," seems equally pertinent today, for if it was fair to reject all previous hypotheses because they could not account for angular momentum and other data of the solar system, it is equally fair to reject all present-day hypotheses for the same reasons.

The dictum of Russell, Dugan, and Stewart (*3*) written in 1926 is still true; namely, that no one has ever imagined a process for accounting for angular momentum of the solar system in evolutionary hypotheses. Ter Haar (*4*), in 1948, referring to von Weizsäcker's dust-cloud hypothesis, wrote: "It is, however, difficult to see why this separation of the cloud according to angular momentum and atomic weight should take place. Also, this process cannot decelerate the sun sufficiently; there is a discrepancy factor of 100,000." Again, in 1951, ter Haar (*5*) wrote: "I would like to stress . . . the difficulty is the slow rotation of the sun. One has to find a mechanism which slows down solar rotation."

Whipple (*6*) wrote in 1952: "The general tendency at present . . . is to ignore the whole problem . . . of angular momentum."

Another problem that seems to present insuperable difficulties is the loss of material from the condensing earth. For example, if the earth contains a ton of hydrogen, obviously the protoplanet contained at least a ton of hydrogen; and if the protoplanet contained more than a ton, there must have been some process by which the excess hydrogen was lost during the formation of the earth. Likewise, similar processes must have existed for every element and isotope in the earth. Computations indicate that such processes are nonexistent, especially for elements with large atomic weights. To illustrate: Urey (*7*) specifically states that there was a loss of Hg during the formation of the earth; however, since the thermal dissipation of Hg requires temperatures that exist only in stellar depths, it follows that the loss of Hg did not occur.

Computations on the excess mass in the protoplanets are based on Kuiper's dust-cloud hypothesis (*8*). Appropriate modifications should be made in numerical values if another dust-cloud hypothesis is used. The excess mass in the protoplanets varies from 14,000 times the mass of the present planet in the case of Mars to 9 times in the case of Jupiter; and the total excess mass in the protoplanets is 50–60 times the mass of the planets.

The loss of 1200 times the mass of the earth from the terrestrial protoplanet with velocities ranging from an original 0 to ~ 30 km sec^{-1} seems impossible, particularly in the case of Ne. To prevent the forming earth from acquiring more than its very small concentration of Ne from a cloud of "cosmic" composition requires that the earth condensed at a temperature of more than 6000° K. At this temperature all compounds are dissociated. and, consequently, all elements with an atomic weight of 20.2 or less, as well as a large proportion of elements with somewhat greater atomic weights, would be lost from the forming earth. Hence, the chemical retention of terrestrial elements suggested by Brown and Patterson (*9*) could not have occurred. There seems, moreover, to be no process with sufficient energy to have kept the forming earth at 6000° K during the time interval required for condensation. There must therefore have been some mechanism to prevent contact of the forming earth with Ne, for it is unthinkable that any sorption of the cosmically abundant Ne could be so inefficient as to result in the meager terrestrial concentration of Ne.

If the planetary bodies condensed from a cosmic cloud, their observed variation in the direction of rotation and revolution seems preposterous. This variation is from 0 to 98°. Imagine, if you can, the mud from a rear bicycle wheel missing the back of the small boy riding through the mire!

The existence of satellites presents another difficulty. The diversity in composition of the mantle of the earth, especially the existence of continents, seems incompatible with any condensation process. But why extend the list? For, obviously, if the condensation of the planets is an imaginary event, most observational data will necessarily be contrary to conclusions drawn from that fantasy. Mass, composition of planetary bodies, energy, momentum, and angular momentum are too real to be derived from folk tales, and

hypotheses that fail to explain so many facts or to conform with so many basic principles are no more entitled to acceptance than folk tales.

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CORRECTION: In the short paper by Heston and Schneiderman that appeared on page 109, SCIENCE, January 30, there is an error at about the middle of column 2. The chemical name for the mustard oil should be "allyl iso-thiocyanate" and not "ethyl iso-thicyanate," as printed. This is our error.

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Book Reviews

The Yeasts: A Taxonomic Study. J. Lodder and N. J. W. Kreger-Van Rij. Amsterdam: North-Holland Pub.; New York: Interscience, 1952. 713 pp. Illus. $19.80.

This single volume of 713 pages is a condensed and revised translation of three earlier volumes: *Die sporogenen Hefen* (1931), *Die anaskosporogen Hefen*, I Hälfte (1934), and *Die anaskosporogen Hefen*, II Hälfte (1942). It also includes the family of Sporobolomycetaceae, which was not covered in the previous editions. Examinations are reported on 1317 strains of yeast.

There are seven chapters in the book and an author and culture name index. A short introductory chapter defines the term "yeasts" and outlines the scope and intent of the book. Chapter II describes 6 morphological and 12 physiological properties that are applied in the classification. Additional properties not previously considered are the formation and shape of the ballistospores and sugar assimilation. There is an interesting discussion of why additional physiological properties, like the assimilation of certain carbon, nitrogen, and sulfur compounds, as well as the vitamin requirements of yeasts, applied by various investigators, were not used.

Chapter III surveys the different types of variation that may occur in yeasts and discusses their significance in relation to taxonomy. Chapter IV has the main line of the classification under the ascosporogenous yeasts, family Endomycetaceae, the yeasts producing ballistospores, family Sporobolomycetaceae, and the asporogenous yeasts, family Cryptococcaceae. The last three chapters discuss the species, and each has a key to the various genera belonging to the three families.

This book on the taxonomy of yeasts is a very thorough study and a most significant accomplishment. It would appear, however, that more physiological properties need to be considered in order to clear up the confusion that now exists, especially at the species level. The names of many yeast species are listed as synonymous. The authors record 67 strains of yeast belonging to the single *cerevisiae* species and 53 strains belonging to its *ellipsoideus* variety. The latter differentiation is based solely on a slightly greater ratio between the length and width of the cells. A single example will suffice to show that these yeasts are not all synonymous but can be differentiated further in some cases. The well-known *cerevisiae* strains Rasse M and Rasse II have similar growth factor requirements but are different in their assimilation of sulfur compounds, whereas the strain Rasse XII is different from both these strains in its growth factor pattern.

This book is highly recommended not only for those mainly concerned with the identification of yeasts but for anyone interested in the ever-expanding and important study of this single-celled organism.

ALFRED S. SCHULTZ

Fleischmann Laboratories, Standard Brands Incorporated
New York

Livestock Health Encyclopedia. The control of diseases and parasites in cattle, sheep and goats, swine, horses and mules. Rudolph Seiden, Ed. and Compiler. New York: Springer Pub., 1951. 614 pp. $7.50.

Into *Livestock Health Encyclopedia*, the author has packed an immense amount of information, which makes it useful to farmers and their advisers, and as a college text for students of medicine and pharmacology. By using a system of symbols and abbreviations, which are translated in two pages at the back of the book, this condensation of knowledge is achieved. The plan does not detract from the reading, in this reviewer's opinion. Whether it will confuse farmers or will be too technical for them remains to be seen.

Allied fields—i.e., wood preservation—for which drugs are used on the farm, are covered, as well as animal health. With so much information packed into 614 pages, there is but little space to tell readers "how to do it," but it is not a how-to-do-it book—

rather, it is an encyclopedia of 3700 entries, illustrated with more than 300 pictures. The whole book is an index; there is no need for a separate one. A bibliography of 9 pages is included.

A copy of this book on every farmer's bookshelf would go far toward bolstering the health and productivity of America's livestock.

LEON F. WHITNEY

Oakwood Road
Orange, Connecticut

Prism and Lens Making. 2nd ed. F. Twyman. London: Hilger and Watts, 1952. (U. S. distrib.: Jarrell-Ash, Boston.) 629 pp. $11.25.

The first edition of this book was published in 1945 and went through three impressions. The present edition has been considerably enlarged and improved. The restriction of the first edition to the methods employed in the famous optical shops of Adam Hilger has been abandoned, and the new edition discusses contributions of other firms as well. Furthermore, several sections have been added covering new fields of development advanced during the past decade.

Most of the book has been written by F. Twyman himself, who devoted his whole life to the solution of practical problems in precision optics. Although the book is intended to serve mainly for the information of optical glassworkers, it is of great interest and value to all scientists who employ optical precision instruments in their research.

The book contains a wealth of expert information about such topics as the nature of grinding, tools and materials, optical glasses, including the new glasses developed in the research laboratory of Eastman Kodak Co., and the artificial crystalline materials for infrared research. Two fascinating chapters deal with testing of optical work and with the application of interferometric methods for testing and correcting lenses and prisms. The last technical chapter treats problems encountered in the making of large objectives and mirrors. Here one also finds notes on the 200-inch mirror on Mount Palomar. The text is elucidated by 260 excellent figures and tables.

K. W. MEISSNER

Department of Physics, Purdue University

The Evolution of Chemistry: A History of Its Ideas, Methods, and Materials. Eduard Farber. New York: Ronald Press, 1952. 349 pp. Illus. $6.00.

"Science looks forward," as Dr. Farber points out in this excellent book, and so many a promising young chemist, diligently pursuing the discipline, finds no time to look backwards. He makes a grave, but understandable, mistake.

It is wholesome to remember the difficulties and handicaps that the pioneers overcame in laying the solid foundations upon which we build today so swiftly and so proudly. But quite aside from this encouragement to a becoming modesty, a comprehensive grounding in the philosophy of chemistry helps us mightily to develop powers of correlation and generalization. Without these two, no chemist rises to the greatest heights in laboratory or schoolroom, in plant or front office.

Dr. Farber has written no text of chemical history, but a most useful and stimulating book for supplementary reading. His theme of how one thought inspires another, one fact points to another, is admirably sustained and aptly illustrated. He fits together the jigsaw of chemical progress into a splendidly composed, easily comprehended picture. I should like to have the economic environment in which the great chemical discoveries were made delineated more clearly and to have had those discoveries carried through more often to their commercial applications. This is, I suspect, but evidence of my own interests.

Of this I am certain—no one after two years of chemistry (needed to read this volume intelligently) and before reaching the ripe age of forty (when life begins) can read this book without becoming Dr. Farber's everlasting debtor.

WILLIAMS HAYNES

Stonecrop Farm
Stonington, Connecticut

Scientific Book Register

Metal Data. Rev. ed. of *Metals and Alloys Data Book.* Samuel L. Hoyt. New York: Reinhold, 1952. 526 pp. Illus. $10.00.

Indiana Scientists: A Biographical Directory and an Analysis. Stephen Sargent Visher *et al.* Indianapolis: Indiana Academy of Science, c/o The State Library, 1951. 286 pp.

Work Measurement: New Principles and Procedures. Adam Abruzzi. New York: Columbia Univ. Press, 1952. 290 pp. $6.00.

High Speed Photography: Its Principles and Applications. George A. Jones. New York: Wiley, 1952. 311 pp. Illus. $6.50.

Automation: The Advent of the Automatic Factory. John Diebold. New York–Toronto: Van Nostrand, 1952. 181 pp. $3.00.

A Television Policy for Education. Proceedings of the Television Programs Institute held under the auspices of the American Council on Education at Pennsylvania State College, April 21–24, 1952. Carroll V. Newsom, Ed. Washington, D. C.: American Council on Education, 1952. 266 pp. Illus. $3.50.

Soluble Silicates: Their Properties and Uses. Vol. 2, ***Technology.*** American Chemical Society Monograph #116. James G. Vail, with assistance of John H. Wills. New York: Reinhold, 1952. 669 pp. Illus. $15.00.

Ferroelectricity. Investigations in Physics, Study No. 1. Eugene Wigner and Robert Hofstadter, Eds. E. T. Jaynes. Princeton, N. J.: Princeton Univ. Press; London: Geoffrey Cumberlege, Oxford Univ. Press, 1953. 137 pp. Illus. $2.00.

Conformal Mapping. L. Bieberbach; trans. from 4th ed. of *Einführung in die Konforme Abbildung* by F. Steinhardt. New York: Chelsea, 1953. 234 pp. Illus. $2.25.

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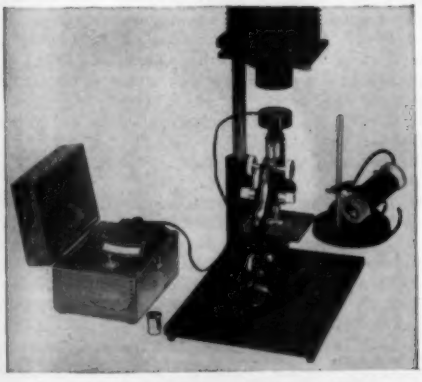

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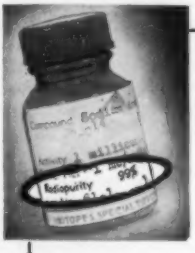

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Publications Received

Alcoholism, 1941–1951. A Survey of Activities in Research, Education and Therapy. Yale Center of Alcohol Studies. New Haven: Quart. J. Studies on Alcohol, 1952. 91 pp. $1.00.

The Anthropology of Iraq. Papers of the Peabody Museum, Vol. XLVI, Nos. 2 and 3. Henry Field. Part II, No. 2, *Kurdistan*. Part II, No. 3, *Conclusions*. Cambridge, Mass.: Peabody Museum. 1952. ix + 174 pp. + 76 pls. $6.85.

Buigingsverschijnselen in Het Nederlands. Deel III. P. Gerlach Royen. Verhandel. Koninkl. Ned. Akad. van Wetenschapp., Afdel. Letterkunde. Nieuwe Reeks Deel LVIII, No. 1. Amsterdam: North-Holland Pub., 1952. 303 pp.

Contributions to the Flora of Venezuela. From Droseraceae through Umbelliferae. Fieldiana: *Botany*, Vol. 28, No. 2. Julian Steyermark *et al.* Chicago: Chicago Natural History Museum, 1952. 195 pp. Illus. $4.00.

Ethnobotany of the Ramah Navaho. Reports of the Ramah Project, Report No. 4. Peabody Museum Papers, Vol. XL, No. 4. Paul A. Vestal. Cambridge, Mass.: Peabody Museum of American Archaeology and Ethnology, Harvard University, 1952. 94 pp. $2.50.

The Feeding Mechanism of a Deep Sea Fish. Chauliodus sloani Schneider. V. V. Tchernavin. London: British Museum (Natural History), 1953. viii + 101 pp. Illus. £1 10s.

Ferns of Hawaii National Park. *Hawaii Nature Notes*, spec. issue, Vol. V, No. 1, June 1952. Douglass H. Hubbard. Hawaii: Hawaii National Park and Hawaii Natural History Association. 40 pp. Illus. 50¢.

Flora of West Virginia. Pt. 1. West Virginia University Bull., Series 52, No. 12–2. P. D. Strausbaugh and Earl L. Core. Morgantown, W. Va.: West Virginia University, 1952. 273 pp. Illus. $1.00.

Flore et Végétation de l'Eqe Groenland. Actualités Scientifiques et Industrielles 1180. H. de Lesse. Expéditions Polaires Françaises. Missions Paul-Emile Victor II. Paris: Hermann & Cie., 1952. 142 pp. Illus.

XLVIII. ***Jahresbericht des Sonnblick-Vereines für das Jahr 1950.*** F. Steinhauser, Ed. Vienna: Springer Verlag, 1952. 38 pp. Illus. 95¢.

Paleographic and Geologic History of Earth Processes. Condensed ed. Ralph L. Caine. Palm Desert, Calif.: Desert Magazine Press, 1952. 89 pp. Illus.

The People Speak. Excerpts from Regional Public Hearings on Health. Vol. 5, *Building America's Health*. President's Commission on the Health Needs of the Nation. Washington, D. C.: GPO, 1953. 521 pp. $2.50.

Philosophy and Psycho-analysis. (Reprints). John Wisdom. New York: Philosophical Library, 1953. 282 pp. $5.75.

Publications of the Dominion Astrophysical Observatory. Vol. IX, No. 4, *The Spectroscopic Orbits of H. D. 100018 (A.D.S. 8189)*. R. M. Petrie and Dorothy M. Laidler. 8 pp. Illus. 25¢. No. 5, *A Spectrographic Study of the Eclipsing Binary 32 Cygni*. K. O. Wright. 30 pp. Illus. 25¢. *The Radial-Velocity Variations of ζ Tauri*. Anne B. Underhill. 18 pp. Illus. Victoria, B. C.: Dept. Mines and Technical Surveys, 1952.

Report of the Committee on the Measurement of Geologic Time. 1951–1952. Div. Geol. Geogr., NRC, Pub. 245. Washington, D. C.: National Acad. Sciences, 1953. 151 pp. Mimeo. $1.50.

Meetings & Conferences

Mar. 8–11. American Institute of Chemical Engineers. Buena Vista Hotel, Biloxi, Miss.

Mar. 9–11. North American Wildlife Conference. Washington, D. C.

Mar. 11–13. National Agricultural Chemicals Association (Spring). Jung Hotel, New Orleans.

Mar. 11–Apr. 8. Commission for Synoptic Meteorology of the World Meteorological Organization. Washington, D. C.

Mar. 12–25. Commission for Climatology of the World Meteorological Organization. Washington, D. C.

Mar. 13–14. Alabama Academy of Science, Inc. Florence.

Mar. 15–19. American Chemical Society (Annual). Hotels Biltmore and Statler, Los Angeles.

Mar. 16–18. Ciba Foundation Symposium on Preservation of Normal Tissues for Transplantation. London.

Mar. 16–20. National Association of Corrosion Engineers (Annual). Hotel Sherman, Chicago.

Mar. 17–19. American Meteorological Society, Symposia on Numerical Prediction; Climatology; Long-Range Forecasting; Ice Island. Atlantic City.

Mar. 18–20. National Health Council (Annual). New York.

Mar. 19–21. Optical Society of America (Spring). Hotel Statler, New York.

Mar. 23–25. American Congress on Surveying and Mapping (Annual). Hotel Shoreham, Washington, D. C.

Mar. 23–25. Conference on Placement of Severely Handicapped. Washington, D. C.

Mar. 23–26. American Association of Petroleum Geologists, Society of Economic Paleontologists and Mineralogists, and Society of Exploration Geophysicists (Joint Annual). Coliseum, Houston.

Mar. 23–26. Institute of Radio Engineers (Annual). Waldorf-Astoria Hotel and Grand Central Palace, New York.

Mar. 23–27. Conference on Tropical Architecture. University of London, England.

Mar. 23–27. Western Metal Congress and Exposition. Pan-Pacific Auditorium, Los Angeles.

Mar. 25–27. American Association of Anatomists (Annual). Ohio State University, Columbus.

Mar. 25–27. American Power Conference (Annual). Sherman Hotel, Chicago.

Mar. 26–28. American Academy of Arts and Sciences. Boston.

Mar. 26–28. American Physical Society. Durham and Chapel Hill, N. C.

Mar. 26–28. Eastern Colleges Science Conference. New York State College for Teachers, Albany.

Mar. 27. Association for Research in Ophthalmology, Midwestern Section. Oscar Johnson Institute, St. Louis.

Mar. 29–Apr. 1. Society of German Chemists, Union of Austrian Chemists, and Swiss Chemical Society (Chemical Meeting). Innsbruck.

Mar. 30–31. Symposium on Retinal Diseases. Department of Ophthalmology, College of Medicine, State University of Iowa, Iowa City.

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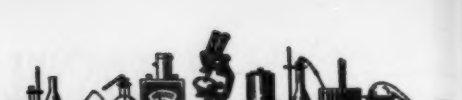

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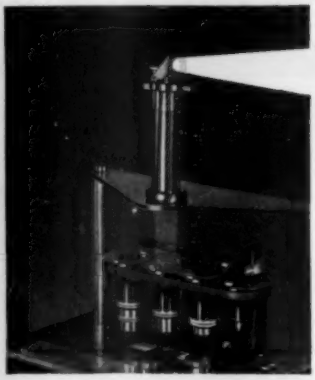

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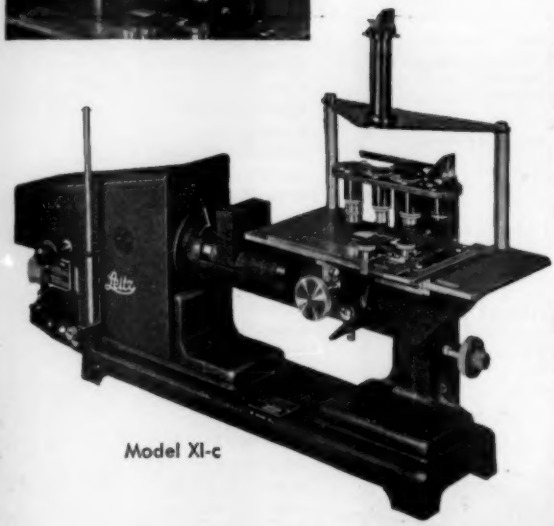

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EATON MANUFACTURING CO.
E. I. DU PONT DE NEMOURS & CO.
FRANKFORD ARSENAL
GENERAL DROP FORGE CO.
GENERAL ELECTRIC CO.
GIBSON REFRIGERATOR CO
GLENN L. MARTIN CO.
HARNISCHFEGER CORP.
HUGHES GUN CO.
INTERNATIONAL HARVESTER
JOLIET ARSENAL
KAISER FRASER
LACLEDE STEEL CO.
ATOMIC ENERGY COMM.
METAL CARBIDES CORP.
MOE LIGHT CO.
OHIO STEEL FOUNDRY
OLDSMOBILE DIVISION, GMC
PICATINY ARSENAL
RHEEM MFG. CO.
UTICA DROP FORGE CO.
VANDERBILT UNIVERSITY
WESTERN CARTRIDGE CO.
WESTERN GEAR WORKS
WILLYS-OVERLAND MOTOR CO.
WRIGHT AERONAUTICAL